PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM

A Dissertation submitted to

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

RASHID.K

REGISTRATION No.: 261425666

Under the guidance of

Dr.C. SENTHIL KUMAR,M.Pharm, PGDCR., PhD

Lecturer

Department of Pharmacology

RVS COLLEGE OF PHARMACEUTICAL SCIENCE

SULUR, COIMBATORE,

TAMILNADU

OCTOBER 2016

DEDICATED TO

ALMIGHTY, FAMILY, GURUS, FRIENDS

AND PROFESSION

DECLARATION

I hereby declare with immense pleasure and satisfaction that this dissertation work entitled" **"PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM"** was carried out by me under the guidance of **Dr. C.SENTHIL KUMAR, M.Pharm, PGDCR., PhD,** Lecturer, Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore.

RASHID. K

Reg.No: 261425666

II. M.Pharm

Department of Pharmacology

R.V.S College of Pharmaceutical Sciences.

Sulur, Coimbatore-641402.

Date:

Place: Coimbatore.

CERTIFICATE

This is to certify that the dissertation work entitled **"PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM"** is a bonafide work of **RASHID.K (Reg No: 261425666)** carried out under my guidance in partial fulfillment for the award of degree of Master of Pharmacy in Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, affiliated to The Tamilnadu Dr. M.G.R Medical University, Chennai.

Dr. C. SENTHIL KUMAR, M.Pharm, PGDCR., PhD
Lecturer
Department of Pharmacology
RVS College of Pharmaceutical Sciences
Sulur, Coimbatore-641402.

Date:

Place: Coimbatore

CERTIFICATE

This is to certify that the dissertation work entitled **"PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM"** is a bonafide work of **RASHID.K (RegNo:261425666)** carried out under the guidance of **Dr.C.SENTHIL KUMAR,** M.Pharm, PGDCR., PhD in partial fulfillment for the award of degree of Master of Pharmacy in Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, affiliated to The Tamilnadu Dr. M.G.R Medical University, Chennai.

Dr. D.BENITO JOHNSON, M. Pharm, Ph.D
Professor and Head
Department of Pharmacology
RVS College of Pharmaceutical Sciences
Sulur, Coimbatore.

Date:

Place**:** Coimbatore

CERTIFICATE

This is to certify that the dissertation work entitled **"PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM"** is a bonafide work of **RASHID. K (RegNo:261425666)** carried out under the guidance of **Dr. C. SENTHIL KUMAR,** M.Pharm, PGDCR., PhD in partial fulfillment for the award of degree of Master of Pharmacy in Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, affiliated to The Tamilnadu Dr. M.G.R Medical University, Chennai.

Dr. R. VENKATANARAYANAN, M. Pharm, Ph.D

Principal

RVS College of Pharmaceutical Sciences

Sulur, Coimbatore-641402

Date:

Place: Coimbatore

EVALUATION CERTIFICATE

Dissertation work : **"PHARMACOLOGICAL ASSESSMENT OF DUAL HERBAL COMBINATION ON CARDIOVASCULAR SYSTEM"**

Name of the Candidate : **RASHID. K (Reg No:261425666)**

Course of Study : **Master of Pharmacy in Pharmacology**

Institution Name : **RVS College of Pharmaceutical Sciences, Sulur, Coimbatore**

INTERNAL EXAMINER **EXTERNAL EXAMINER**

Date :

Place :

ABSTRACT

The present study designed to investigate the toxicological profile, cardioprotective and in-vitro thrombolytic activity of novel herbal combination with nigella sativa oil and barley water. The acute and sub-acute (28 days) oral toxicity of herbal combination on wister rats was performed. All the morphological, hematological, biochemical and hostological changes in addition to mortality and body weight changes were recorded. The acute toxicity result was shown no significant toxicity and mortality observed in the both sexes of animals upto maximum dose. Hence it may be recommend for clinical utility.

The sub-acute toxicity result shown no dose related toxicity changes was seen all groups. Hence it can be used for chronic treatment.

Myocardial infarction was induces by the administration of isoproterenol hydrochloride (ISO) 85 mg/kg, i.p, the rats were pretreated with the dual herbal combination through the oral route. ISO alone, treated rats showed the elevated serum cardiac biomarkers such as LDH, CK-MB, SGOT and SGPT due to myocardial damage produced by ISO. This further confirmed by histopathological examination of heart tissues. Oral administration of dual herbal combination significantly restored the level of serum cardiac markers and other altered biochemical parameters.

An in-vitro thrombolytic model was used to check the clot lysis effect of the the prepared dual herbal combination, streptokinase was used as a positive control and water as negative control. The study suggests that thrombolytic activity of dual herbal combination could be considered as very promising and beneficial for therapeutic purposes

Key words: Black seed oil, *Hordeum Vulgare,* acute and sub-acute toxicity, cadioprotection, thrombolysis

ACKNOWLEDGEMENT

First of all, I would like to extend my sincere gratitude to God *Almighty*, whose divine intervention was instrumental in the successful completion of this project work. There are a number of people whom I consider of this simple anthology and to whom I owe so much.

I would like to express my whole hearted gratitude to **My Parents**, **My sisters** and **Brothers** without whose support and blessings, this endeavor would not have been completed.

It is a great pleasure to express my gratitude and sincere thanks to my beloved guru and my respected guide **Dr. C. SENTHIL KUMAR, M.Pharm, PGDCR., PhD** Lecturer, department of pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, for his invaluable guidance, help, suggestions and the confidence that he has shown in me throughout the course of my work.

I sincerely and honestly expressing my salute and inexpressible warm thank to **Dr. D. BENITO JOHNSON, M.Pharm, Ph.D, Head of the Department,** RVS College of pharmaceutical sciences, Sulur, Coimbatore, for his help and guidance throughout the completion of my work.

I sincerely thank **Dr. R. VENKATANARAYANAN, M.Pharm., Ph.D, Principal**, RVS College of pharmaceutical sciences, Sulur, Coimbatore, for his inspirations and being a great facilitator.

I am very thankful to **Dr. R..MANAVALAN. M.Pharm.,Ph.D., FICS.,FIC**., Professor of pharmaceutics, Head &Research Director RVS College of pharmaceutical sciences, Sulur, Coimbatore, for his inspirations and being a great facilitator.

I am grateful to **Mr. SURESH, Mr. E. ABRAHAM, Miss. C. KAMALESHWARI, Mr. SAKTHIVEL, Miss. ASHWINI, Mrs. DHANAM** and all the teachers and non teachers of my college for their meticulous guidance and encouragement provided to me for the completion of my dissertation work.

However, it would not have been possible to complete my work without kind support and help of all my friends especially **SHRUTI RAMESH TIMANE, MUHAMMED HALEEL.P.M, SANDEEP.M, SABITH.S, ROLLY ROY, ASHIF, MUFEEDA, BANSHONGDOR H MAWLIEH AND TIMAI PASSAH.**

I would like to extend my sincere thanks to each and every individual who have been a source of support and helped me to complete my dissertation work successfully.

Once again I express my thank to almighty and one and all whom I might have missed to mention who gave constant encouragement and help throughout my educational career

RASHID.K

LIST OF ABBREVIATIONS

1	AA	AMINO ACIDS
2	ACE	ANGIOTENSIN CONVERTING ENZYME
3	AMI	ACUTE MYOCARDIAL INFARCTION
4	ADP	ADINOSIN DIPHOSPHATE
5	AST,	ALANINE AMINOTRANSFERASE
6	ALT	ALANINE AMINOTRANSFERASE
7	API	ACTIVE PHARMACEUTICAL INGREDIENTS
8	BW	BARLEY WATER
9	CAT	CATALASE
10	CCL4	CARBON TETRACHLORIDE
11	CPCSEA	COMMITTEE FOR PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS
12	MG	MILLIGRAM
13	MIN	MINUTE (S)

14	KG	KILOGRAM
15	COX	CYCLOXYGENASE
16	LOX	LIPOXYGENASE
17	CK-MB	CREATINE KINASE
18	CVDS.	CARDIOVASCULAR DISEASES OR CEREBROVASCULAR DISEASE
19	DVT	DEEP VENOUS THROMBOSIS
20	GST	GLUTATHIONE-S-TRANSFERASE
21	HDL	HIGH DENSITY LIPOPROTEIN
22	HB	HEMOGLOBIN
23	WBC	WHITE BLOOD CORPUSCLE
24	RBC	RED BLOOD CORPUSCLE
25	SGPT	SERUM GLUTAMIC PYRUVIC TRANSAMINASE
26	SGOT	SERUM GLUTAMIC OXALOACETIC TRANAMINASE
27	IAEC	INSTITUTIONAL ANIMAL ETHICAL COMMITTEE

28	ISO	ISOPROTERENOL
29	ISPH	ISOPROTERENOL HYDROCHLORIDE
30	LDH	LACTATE DEHYDRO-GENASE
31	LD	LEATHAL DOSE
32	LDL	LOW DENSITY LIPOPROTEIN
33	MDA	MALONDIALDEHYDE
34	MCH	MEAN CORPUSCULAR HEMOGLOBIN
35	MCHC	MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION
36	MCV,	MEAN CORPUSCULAR VOLUME
37	MI	MYOCARDIAL INFARCTION
38	MPV	MEAN PLATELET VOLUME
39	NADPH	NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
40	NSE	NIGELLA SATIVA EXTRACT
41	NSO	NIGELLA SATIVA OIL

42	OECD	ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
43	PCV	PACKED CELL VOLUME
44	KBRO3	POTASSIUM BROMATED
45	RDW	RED CELL DISTRIBUTION WIDTH
46	SD	STANDARD DEVIATION
47	SEM	STANDARD ERROR MEAN
48	SK	STREPTOKINASE
49	TQ	THYMOQUINONE
50	TG	TRIGLYCERIDES
51	VLDL	VERY LOW DENSITY LIPOPROTEIN
52	WHO	WORLD HEALTH ORGANIZATION

LIST OF FIGURES

SL. NO.	FIGURE	PAGE NO
1	THE CIRCULATORY SYSTEM.	2
2	HISTOLOGICAL ORGANIZATION OF CARDIAC MUSCLE TISSUE.	4
3	DISTRIBUTION OF MAJOR CAUSES OF DEATH INCLUDING CVDS	5
4	SCHEMATIC OF SEQUENTIAL PROGRESSION OF CORONARY ARTERY LESIONS	6
5	PATHOPHYSIOLOGY OF MYOCARDIAL INFARCTION.	7
6	ACUTE MYOCARDIAL INFARCT, PREDOMINANTLY OF THE POSTEROLATERAL LEFT VENTRICLE.	8
7	ISOPROTERENOL INDUCED MYOCARDIAL DAMAGE	10
8	SPECIFIC BLOOD PROTEIN LEVELS THAT LEAK OUT OF FATALLY INJURED MYOCYTES.	12
9	RELEASE OF CARDIAC MARKERS AFTER ACUTE MYOCARDIAL INFARCTION (AMI).	13
10	THE HAEMOSTATIC PLUG FORMATION	17
11	THROMBUS FORMATION	17
12	VIRCHOW'S TRIAD IN THROMBOSIS	28
13	THROMBUS IN THE LEFT AND RIGHT VENTRICULAR APICES	20

14	NIGELLA SATIVA FLOWER AND SEED.	28
15	DIFFERENT CHEMICAL COMPONENTS OF NIGELLA SATIVA	29
16	BARLEY PLANT AND GRAINS	30
17	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON FEED INTAKE IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	54
18	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON WATER INTAKE IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	56
19	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON BODY WEIGHTS IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	57
20	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON RELATIVE ORGAN WEIGHT IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	58
21	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON HEMATOLOGICAL PARAMETERS IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	62
22	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON HEMATOLOGICAL PARAMETERS IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	62
23	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON HEMATOLOGICAL PARAMETERS IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	63
24	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON SERUM BIOCHEMICAL PROFILE IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES).	65

25	GRAPHICAL REPRESENTATION EFFECT OF NSO+BW ON SERUM LIPID PROFILE IN CONTROL AND EXPERIMENTAL RATS (SUB ACUTE TOXICITY STUDIES)	66
26	HISTOLOGICAL SECTIONS OF KIDNEY FROM THE CONTROL AND MAXIMUM DOSE	67
27	HISTOLOGICAL SECTIONS OF LIVER FROM THE CONTROL AND MAXIMUM DOSE	67
28	HISTOLOGICAL SECTIONS OF HEART FROM THE CONTROL AND MAXIMUM DOSE	67
29	HISTOLOGICAL SECTIONS OF BRAIN FROM THE CONTROL AND MAXIMUM DOSE	68
30	GRAPHICAL REPRESENTATION OF EFFECT OF NSO+BW ON MYOCARDIAL MARKER ENZYMES IN ISO- INDUCED MYOCARDIAL INFARCTION.	69
31	HISTOPATHOLOGICAL SECTION OF HEART AFTER TREATMENT PERIOD.	71
32	GRAPHICAL REPRESENTATION OF IN-VITRO THROMBOLYTIC ACTIVITY OF NSO+BW	72

LIST OF TABLES

S.NO	TABLES	PAGE NO
1	PHARMACOTHERAPY OF MYOCARDIAL INFARCTION	15
2	LIST OF THROMBOLYTIC AGENT	22
3	NAME AND SUPPLIER OF HERBAL INGREIENTS	43
4	NAME AND SUPPLIER OF THE CHEMICALS USED	43
5	NAME AND SUPPLIER OF THE INSTRUMENTS USED	44
6	EFFECT OF NSO+BW ON AUTONOMIC OBSERVATIONS	51
7	EFFECT OF COMBINATION OF NSO+BW ON FEED INTAKE IN RATS-SUB-ACUTE TOXICITY STUDY	53
8	EFFECT OF COMBINATION OF NSO+BW ON WATER INTAKE IN RATS-SUB-ACUTE TOXICITY STUDY	54
9	EFFECT OF COMBINATION OF NSO+BW ON BODY WEIGHT GAIN IN RATS-SUB-ACUTE TOXICITY	56
10	EFFECT OF COMBINATION OF NSO+BW ON ORGAN WEIGHT IN RATS-SUB-ACUTE TOXICITY STUDY	57
11	EFFECT OF COMBINATION OF NSO+BW ON HEMATOLOGICAL PARAMETERS IN RATS-SUB-ACUTE TOXICITY STUDY	60
12	EFFECT OF COMBINATION OF NSO+BW ON BIOCHEMICAL PARAMETERS IN RATS-SUB-ACUTE TOXICITY STUDY	64
13	EFFECT OF NSO+BW ON THE LEVEL OF SERUM CARDIAC MARKERS AND IN NORMAL AND ISOPROTERENOL INDUCED MI IN RATS.	67
14	RESULT OF THROMBOLYTIC ASSAY	71

TABLE OF CONTENTS

SL. NO	CONTENTS	PAGE NUMBERS
1.	**INTRODUCTION**	1-31
1.1	HEART AND BLOOD CIRCULATION	1
1.2	STRUCTURE OF THE HEART WALL	3
1.3	CARDIAC MUSCLE TISSUE	3
1.4	CARDIOVASCULAR DISEASES	5
1.5	PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASES	5
1.6	ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION	9
1.7	DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION	11
1.8	LABORATORY EVALUATION	11
1.9	TREATMENT OF MYOCARDIAL INFARCTION	14
1.10	HAEMOSTASIS AND THROMBOSIS	16
1.11	THROMBUS VS. EMBOLUS	18
1.12	TYPES OF THROMBOSIS	18
1.13	VENOUS THROMBOSIS	18
1.14	ARTERIAL AND CARDIAC THROMBOSIS	20
1.15	STROKE	20
1.16	MYOCARDIAL INFRACTION	21

1.17	DIAGNOSIS OF MYOCARDIAL INFARCTION	21
1.18	THROMBOLYTIC AGENTS	22
1.19	THROMBOLYTIC THERAPY IN ACUTE MYOCARDIAL INFARCTION	23
1.20	ADVERSE EFFECTS OF THROMBOLYTIC THERAPY	23
1.21	HERBAL REMEDIES FOR CARDIOVASCULAR DISEASES	23
1.22	CARDIOPROTECTIVE HERBS	24
1.23	HERBAL CARDIOPROTECTIVE COMPOUNDS	25
1.24	NATURAL THROMBOLYTIC REMEDIES	26
1.25	NIGELLA SATIVA: PHARMACOGNOSTIC DESCRIPTION	26
1.26	TAXONOMIC CLASSIFICATION OF NIGELLA SATIVA	27
1.27	SYNONYMS AND HABITAT	27
1.28	MORPHOLOGY OF THE PLANT	28
1.29	NIGELLA SATIVA SEED	28
1.30	ACTIVE CONSTITUENTS	28
1.31	PHARMACOLOGICAL EFFECTS FOR POTENTIAL HEALTHCARE	29
1.32	HORDEUM VULGARE: BOTANICAL DESCRIPTION	29
1.33	TAXONOMIC CLASSIFICATION OF HORDEUM VULGARE	30

1.34	NUTRITIONAL INFORMATION OF HORDEUM VULGARE	31
1.35	PHARMACOLOGICAL EFFECTS FOR POTENTIAL HEALTHCARE	31
2.	**LITERATURE REVIEW**	32-40
3.	**AIM AND OBJECTIVE**	41
4.	**PLANE OF WORK**	42
5.	**MATERIALS AND METHOD**	43-49
5.1	HERBAL COMPONENTS	43
5.2	LIST OF CHEMICALS	43
5.3	LIST OF INSTRUMENTS	44
5.4	EXPERIMENTAL ANIMALS	44
5.5	APPROVAL OF PROTOCOL	44
5.6	**TOXICOLOGICAL EVALUATION**	45
	ACUTE TOXICITY STUDIES	
	SUB- ACUTE TOXICITY STUDIES	
5.7	**PHARMACOLOGICAL STUDIES**	47
	ASSESSMENT OF CARDIOPROTECTIVE ACTIVITY	
	IN-VITRO THROMBOLYTIC ACTIVITY	
5.8	**STATISTICAL ANALYSIS**	49
6.	**RESULTS AND DISCUSSION**	50-76
6.1	**ACUTE TOXICITY STUDY**	50

6.2	**SUB-ACUTE TOXICITY STUDY**	52
6.3	**CARDIAC PROTECTIVE ACTIVITY**	67
6.4	**IN-VITRO THROMBOLYTIC ACTIVITY**	70
7.	**SUMMERY AND CONCLUSSION**	77
8.	**BIBILIOGRAPHY**	78

CHAPTER – I
INTRODUCTION

Every living cell relies on the surrounding interstitial fluid as the major source of oxygen and nutrients and as a place for the disposal of cellular wastes. Levels of gases, nutrients, and cellular waste products in the interstitial fluid are kept stable through continuous exchange between the interstitial fluid and the circulating blood. The blood must stay in motion to maintain the body homeostasis. If blood stops flowing through vessels and tissues, its oxygen and nutrient supplies are exhausted quickly, its capacity to carry wastes is soon reached, and neither hormones nor white blood cells can get to their intended targets. Thus, all of the functions of the cardiovascular system ultimately depend on the heart, because it is the heart that keeps circulation of blood. This muscular organ beats approximately 100,000 times in a day, propelling blood through the blood vessels.

1.1. Heart and Blood Circulation

Despite its impressive workload, the heart is a small organ; your heart is roughly the size of your clenched fist. The heart's four muscular chambers, the right atrium and left atrium and right ventricle and left ventricle, work together to pump blood through a network of blood vessels between the heart and the peripheral tissues. The circular network can be subdivided into two: the pulmonary circuit, which carries carbon dioxide-rich blood (impure blood) from the heart to the gas exchange surfaces of the lungs (alveoli) and returns oxygen-rich blood (pure blood) to the heart; and the systemic circuit, which transports oxygen-rich blood from the heart to the rest of the body's cells, returning carbon dioxide-rich blood back to the heart.

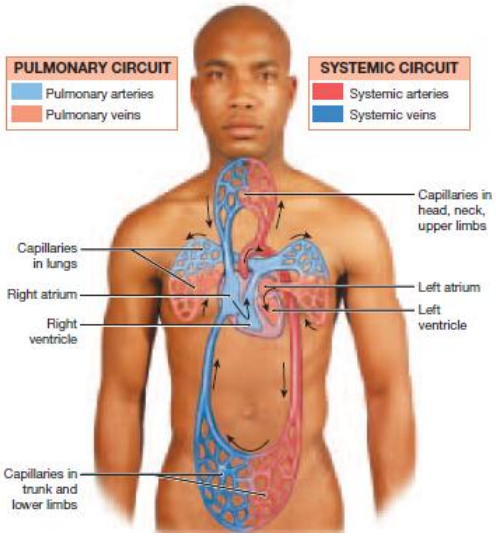


Fig: 1. The circulatory system.

The right atrium receives blood from the systemic circuit, and the right ventricle release blood into the pulmonary circuit. The left atrium collects blood from the pulmonary circuit, and the left ventricle discharges blood into the systemic circuit. When the heart beats, atria contract first, followed by the ventricles. The two ventricles contract at the same time and eject equal quantity of blood into the pulmonary and systemic circuits.

Each circuit begins and ends at the heart. Capillaries are small, thin-walled vessels that interconnect the smallest arteries and veins. Capillaries are called exchange vessels because their thin walls permit exchange of dissolved gases, nutrients and waste products between the blood and surrounding tissues.

1.2. Structure of the Heart Wall

A section along the wall of the heart reveals three distinct layers:

(1) An outer epicardium (visceral pericardium)

(2) A middle myocardium, and

(3) An inner endocardium.

The epicardium or visceral pericardium; it forms the external surface of the heart. The epicardium is a main membrane consisting of a mesothelium covering a supporting layer of areolar connective tissue.

The myocardium contains multiple, interlocking layers of cardiac muscle tissue, with associated connective tissues, blood vessels, and nerves etc. The relatively thin atrial myocardium consists of few layers that form figure-eights as they pass from atrium to atrium. The ventricular myocardium is much thicker than atrial myocardium and the muscle orientation changes from layer to layer. Superficial ventricular muscles wrap around both ventricles; deeper muscle layers spiral around and between the ventricles from the attached *base* toward the free tip, or *apex*, of the heart (Fig: 2)

The inner surfaces of the heart, including the valves, are covered by a simple squamous epitheliumis called the endocardium (en-do- KAR-de-um; *endo-*, inside). The endocardium is continuous with the endothelium of the particular attached blood vessels.

1.3. Cardiac Muscle Tissue

The histological characteristics of cardiac muscle tissue give the myocardium and its unique functional properties. Cardiac muscle cells, or *cardiocytes*, are relatively small, averaging size 10–20 mm in diameter and 50–100 mm in length. A typical cardiocyte has a single nucleus,which is centrally placed (Fig: 2)

Cardiac muscle cells resemble skeletal muscle fibers, although they are much smaller than skeletal muscle fibers in that each cardiac muscle cell contains organized myofibrils and the alignment of their sarcomeres produces striations. However, the cardiac muscle fibrils are differ from skeletal muscle fibers in several important respects:

Cardiac muscle cells are almost totally dependent on aerobic respiration to obtain the needed energy to continue contracting. Hundreds of mitochondria present in the sarcoplasm of a cardiac muscle cell and abundant reserves of myoglobin (to store oxygen). Energy reserves are maintained in the form of glycogen and lipid inclusions.

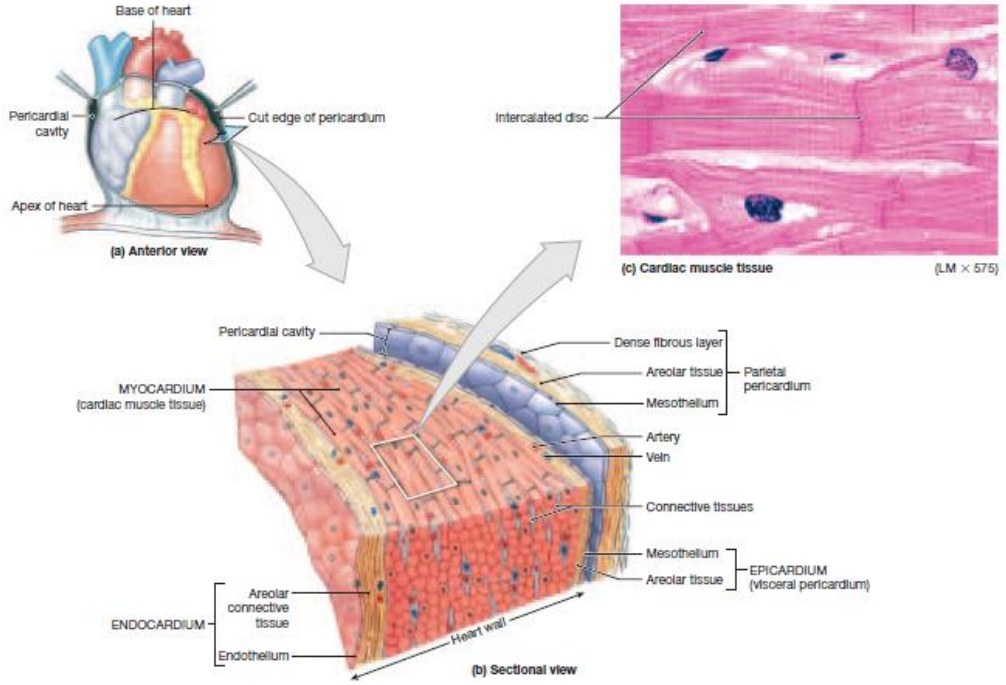


Fig: 2. Histological Organization of Cardiac Muscle Tissue.

The relatively short T-tubules of cardiac muscle cells do not form triads with the sarcoplasmic reticulum. The circulatory supply of cardiac muscle tissue is more extensive even than that of red skeletal muscle fiblils.

1.4. Cardiovascular Diseases

Cardiovascular diseases (CVDs) are group of disorders of the heart and blood vessels and include coronary heart disease (heart attack), cerebrovascular disease, rheumatic heart disease and other conditions. Four out of five cardiovascular disease deaths are due to heart attacks and strokes. Individuals at risk of CVD may demonstrate raised blood pressure, glucose, and lipids as well as overweight and obesity etc[1].

Cardiovascular Diseases are responsible for over 17.3 million deaths per year and are the leading causes of death in the world. Deaths due to heart attacks, strokes and other types of CVDs as a proportion of total cardiovascular deaths for males and females[2] (Fig: 3).

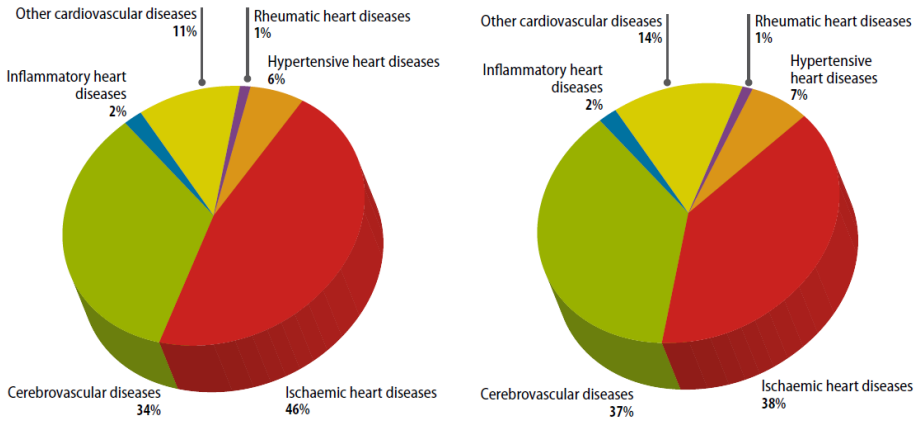


Fig: 3. Distribution of major causes of death including CVDs

1.5. Pathophysiology of Cardiovascular Diseases

Atherosclerosis is the main underlying pathological processes that leads to heart attacks (coronary heart disease) and strokes (cerebrovascular disease). The early changes of atherosclerosis develop in childhood and adolescence due to the overall effect of a many of risk factors[3-5]. They include use of tobacco, physical inactivity, unhealthy diet, use of alcohol, hypertension, diabetes, raised blood lipid level, obesity, poverty, low educational status,

advancing age, male gender, genetic disposition and psychological factors etc.

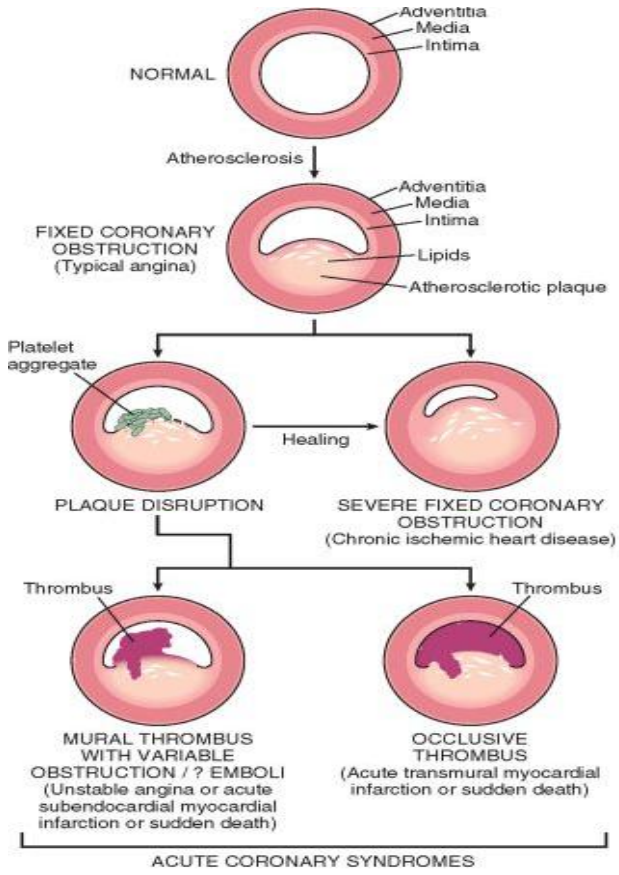


Fig: 4- Schematic of sequential progression of coronary artery lesions

When the process continues, there is thinning of the fibrous cap accompanied by fissuring of the endothelial surface of the formed plaque, which may rupture. From the ruptured plaque, lipid fragments and cellular debris are released into the vessel lumen. These released materials are exposed to thrombogenic agents on the endothelial surface, resulting in the formation of a thrombus. The coronary blood vessel or a cerebral blood vessel is blocked, when the thrombus is large enough, this results in a heart attack or stroke[7, 8].

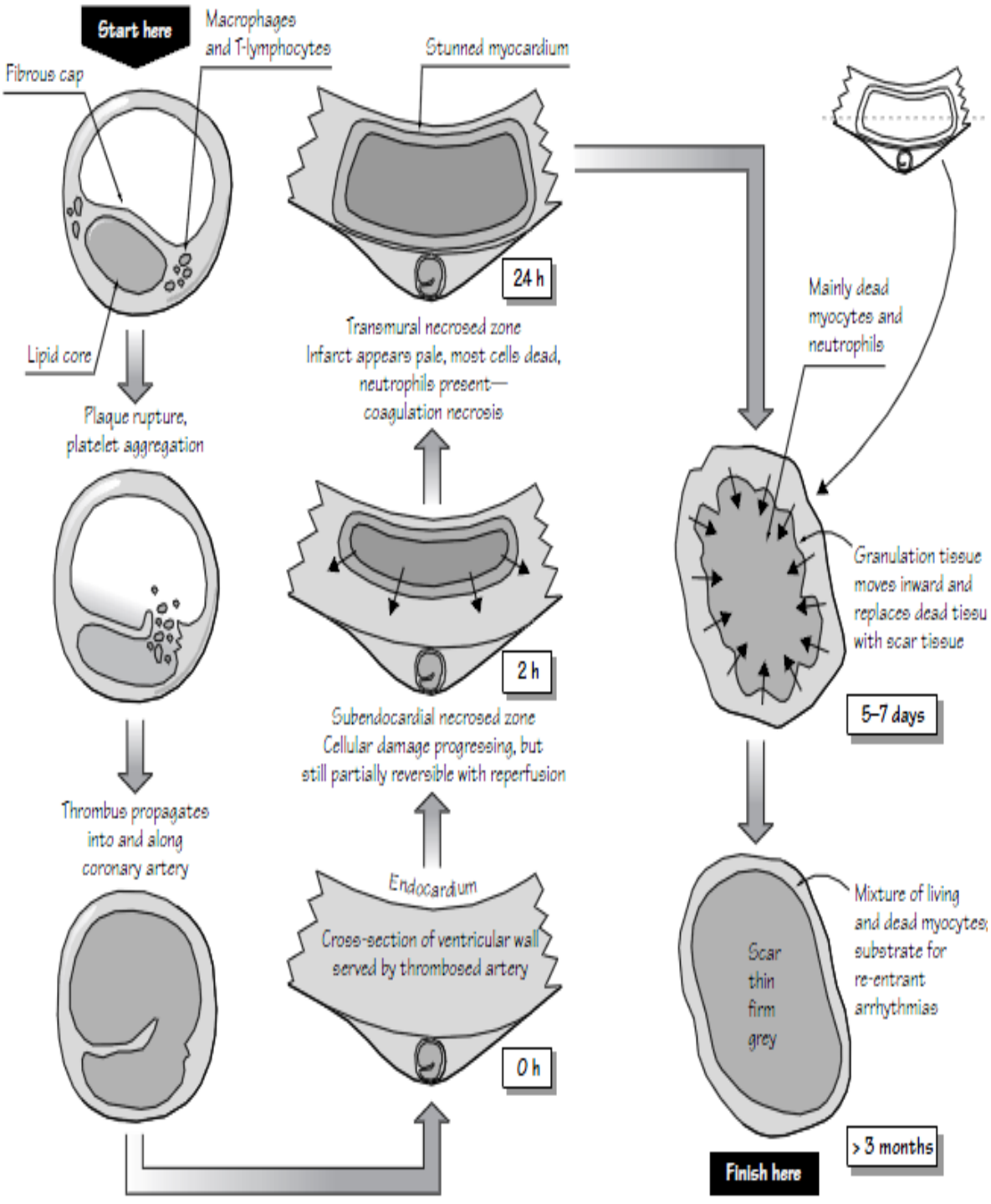


Fig.5: Pathophysiology of myocardial infarction.

Heart attack : When the blood flow to the heart is cut off, due to a thrombus on a ruptured atherosclerotic plaque, the dimished supply of oxygen and nutrients can damage the heart muscle cells, resulting in a heart attack. When the blood flow is decreased due to a blockage, it causes chest pain (angina) due to ischaemia of heart.

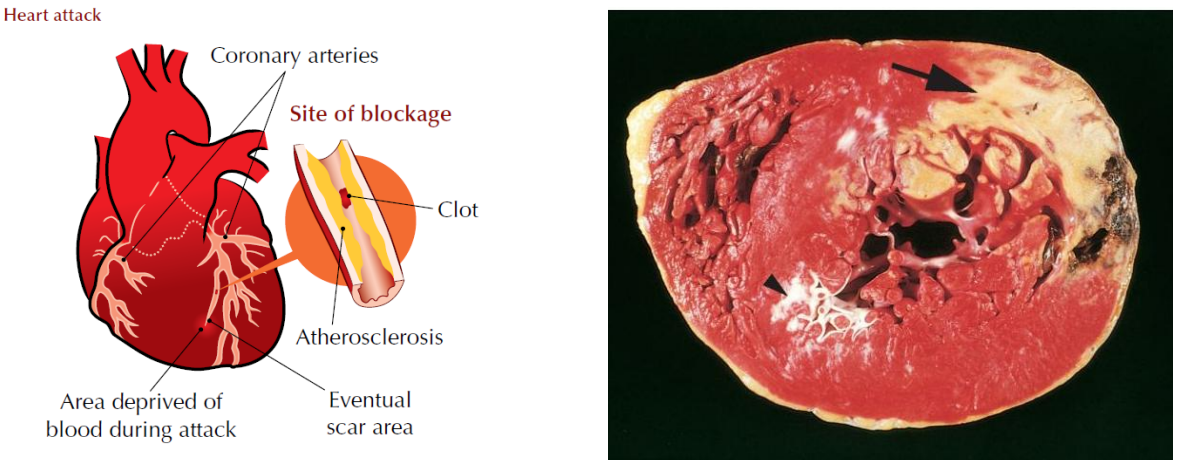


Fig: 6. Acute myocardial infarct, predominantly of the posterolateral left ventricle.

Stroke: The pathophysiology of ischaemic stroke is more diverse and includes, besides thrombus formation in cerebral blood vessels (ischaemic stroke), small vessel disease in the brain linked to vascular risk factors. Another cause of stroke is haemorrhage (bleeding) due to the rupture of a blood vessel because of uncontrolled high blood pressure or atherosclerosis (haemorrhagic stroke). In addition, strokes can also be caused by a travelling blood clot. If a person has an irregular heartbeat, blood clots may form in the heart and travel along with the blood to the brain. A clot carried to the cerebral circulation in this way can be trapped in a cerebral blood circulating route and block the blood flow to an area of the brain.

1.6. Isoproterenol Induced Myocardial Infarction

Isoproterenol hydrochloride (ISPH), a synthetic catecholamine and β-adrenergic agonist that causes severe stress in the myocardium and infarct-like necrosis of the heart muscles[9]. ISPH induces myocardial injury by altering the vascular membrane permeability, which brings about the loss of functions and integrity of myocardial membranes[10].

Isoproterenol-induced myocardial necrosis is considered as one of the most widely used experimental model to study the beneficial effects of many drugs on cardiac function[11]. Catecholamine readily undergoes oxidation and the oxidative products of catecholamine are responsible for the myocardial damage[12]. Catecholamines have been shown to increase myocardial oxygen consumption and enhance the extents of myocardial damage during evolving acute myocardial infarction[13]. Myocardial infarction induced by isoprenaline hydrochloride [isoproterenol; L-β-(3,4-dihydroxyphenyl)-α- isopropylaminoethanol hydrochloride], a β- adrenergic agonist, shows many metabolic and morphologic aberrations in the myocardium of experimental animal's heart is similar to those observed in myocardial infarction in human[14]. When the large dose of catecholamines is administered, particularly isoproterenol hydrochloride to the experimental animal constitutes a rapid and reproducible means of provoking myocardial ischemia (Fig :6).

Isoproterenol-induced MI serves as a well-standardized model because the pathophysiological changes following isoproterenol administration are comparable to those taking place in human heart[15]. Intraperitoneal administration of isoprenaline can produces ischemic lesions and increases lipid peroxidation in the myocardium, which plays a significant part in the pathogenesis of myocardial dysfunction[16]. Alterations in the activities of antiperoxidative enzymes [ie ; superoxide dismutase (SOD) and catalase (CAT)] and glutathione dependent antioxidant enzymes [glutathione peroxidase (GPX) and glutathione-S-transferase (GST)] have been reported in experimentally ISPH induced myocardial infarction in rat model[17]. AMI causes a detectable ris in the plasma concentration of cardiac marker enzymes, which are normally concentrated within cardiac

cells. The enzymes most widely used in the detection of MI are, creatine kinase (CK-MB), aspartate aminotransferase (AST) and lactate dehydro-genase (LDH) etc. An increased level of free radical generating system and malondialdehyde (MDA) and lowered levels of free radical scavenging systems seem to have critical role in the ischemic heart condition[18].

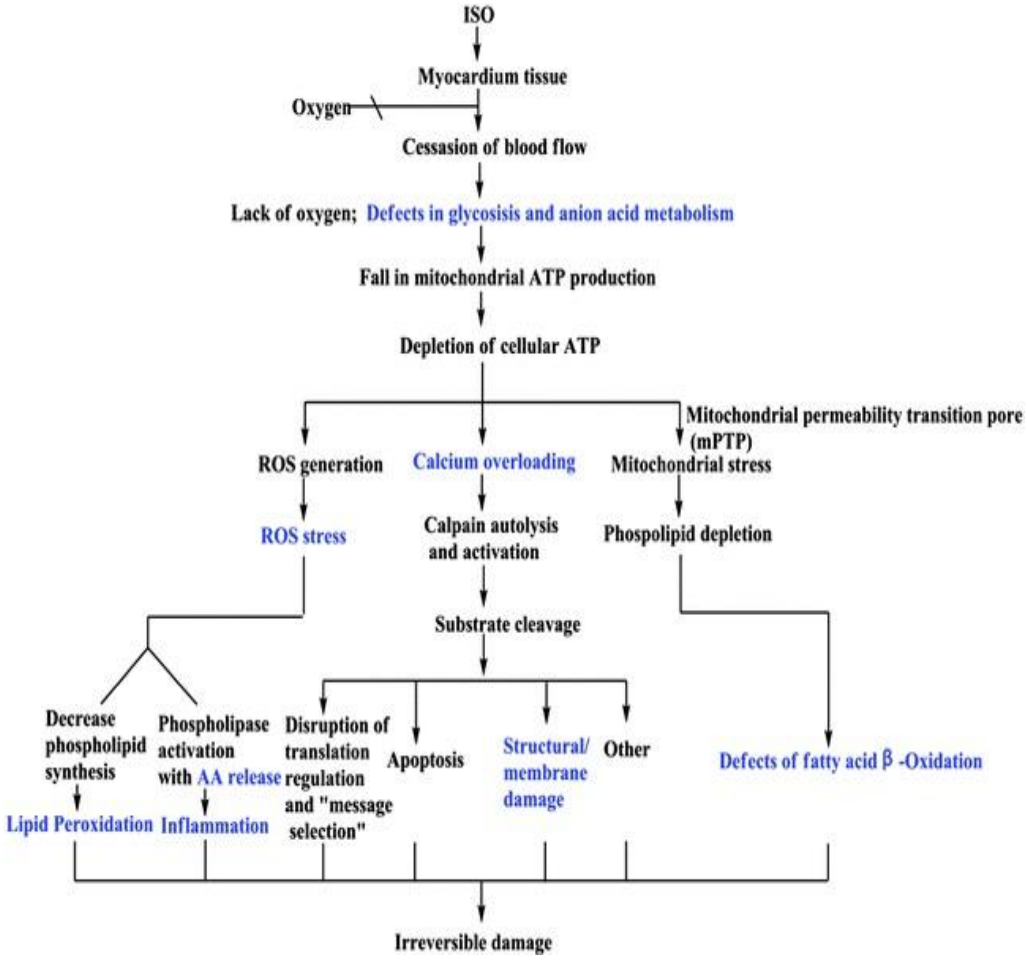


Fig.7: Isoproterenol induced myocardial damage

1.7. Diagnosis of Acute Myocardial Infarction

In the clinical assessment of coronary heart diseases, electrocardiography is an essential adjunct instead of clinical history and physical examination. A proper diagnosis (rapid and accurate) in patients with acute myocardial infarction is vital, as expeditious reperfusion therapy can improve prognosis. ST segment elevation in two or more anatomically contiguous leads is the most frequently used electrocardiographic criterion for identifying acute myocardial infarction. The elevation ST segment associated with an evolving myocardial infarction is often readily identifiable, but a knowledge of the common "pseudo" infarct patterns is essential to avoid the unnecessary use of thrombolytic therapy[19]

Echocardiogram and angiogram with dye (contrast method by using X-ray) also used to assess or get detailed information of the arteries in the heart.

1.8. Laboratory Evaluation

Quantitative or qualitative evaluation of specific blood protein levels that leak out of fatally injured myocytes; these molecules include myoglobin, cardiac troponins T and I, the MB fraction of creatine kinase (CK-MB), lactate dehydrogenase, and many others (Fig: 8)[20]. If the myocardial injury is established when blood levels of these cardiac biomarkers are increased in the clinical setting of acute ischemia. The intracellular location, molecular weight, the blood flow, lymphatic drainage in the area of the infarct, and the rate of elimination of the marker from the blood are some factors which affect the appearance of these markers in the peripheral circulation.

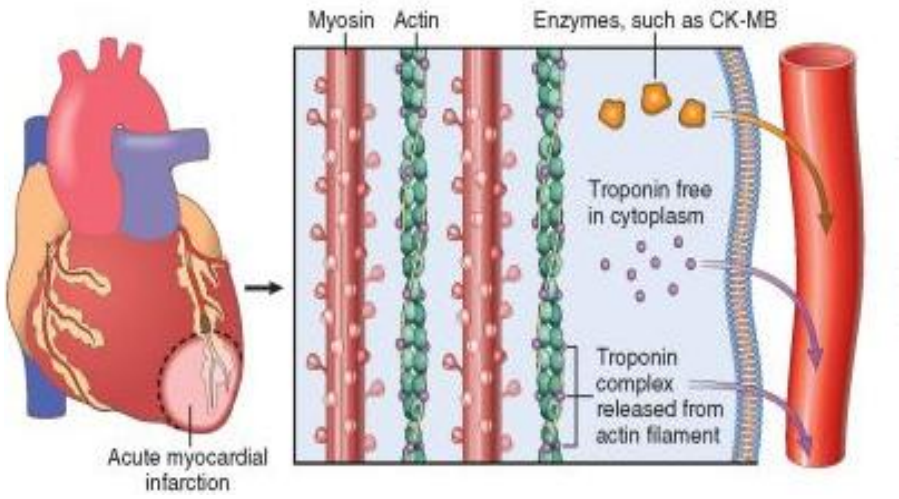


Fig. 8: Specific Blood Protein Levels That Leak Out Of Fatally Injured Myocytes.

Cardiac-specific proteins like *Troponins I and T* (proteins that regulate calcium-mediated contraction of cardiac and skeletal muscle) are the most sensitive and specific biomarkers of myocardial damage .They are not not normally detectable in the circulation.

The level of both proteins begins to rise at 2 to 4 hours and peak at 48 hours once the MI occur. Formerly the "gold standard," cardiac creatine kinase remains useful. Creatine kinase (composed of two isoforms designated "M" and "B") , an enzyme that is present in brain, myocardium, and skeletal muscle. MB heterodimers principally in cardiac muscle, with minute quantity also being found in skeletal muscle. MM and BB are the homodimers found in the cardiac, skeletal muscle, brain, lung, and many other tissues respectively[21].

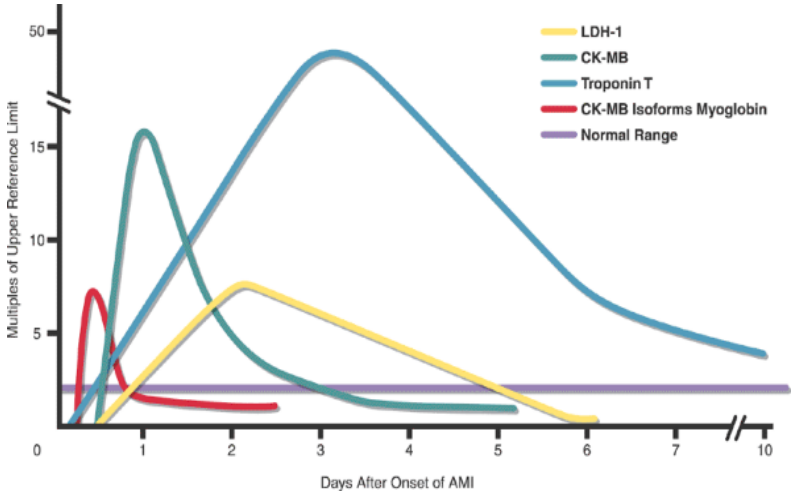


Fig.9: Release of cardiac markers after acute myocardial infarction (AMI).

The rised CK-MB (elevated within 2 to 4 hours of the onset of MI, peaks at about 24 hours) returns to normal within approximately 72 hours. The diagnostic sensitivities of cardiac troponin and CK-MB measurements are similar in the MI in early stage. Elevated troponin levels persist for approximately 7 to 10 days after acute MI, well after CK-MB levels have returned to normal.Troponin and CK-MB levels peak earlier in patients whose hearts are successfully reperfused, because proteins are washed out of the necrotic tissue more rapidly. Unchanged levels of CK-MB and troponins over a period of 48 hours essentially excludes the diagnosis of MI[21].

Following an MI, the levels of Aminotransferases such as Aspartate aminotransferase and Alanine aminotransferase (AST, or SGOT ang SGPT) also elevated in the serum. L-Lactate dehydrogenase is a tetrameric enzyme whose four subunits occur in two isoforms, designated H (for heart) and M (for muscle). Isozymes of lactate dehydrogenase (LDH-1) are used to detect myocardial infarctions[22].

1.9. Treatment of Myocardial Infarction

Medication/medication class	Use	Comments
Antihypertensive agents		
ACE inhibitors	All patients with hypertension, diabetes mellitus, chronic kidney disease, or left ventricular dysfunction	Decrease mortality
Angiotensin receptor blockers	All patients with hypertension, diabetes, chronic kidney disease, or left ventricular dysfunction, and in whom ACE inhibitors are not tolerated	No additional benefit compared with ACE inhibitors; may be considered in combination with ACE inhibitors for heart failure with left ventricular dysfunction
Beta blockers	All patients with history of MI, acute coronary syndrome, or left ventricular dysfunction, unless contraindicated	Decrease mortality; avoid $beta_2$ selective agents and agents with intrinsic sympathomimetic properties
Calcium channel blockers	Patients in whom beta blockers are not tolerated	Avoid short-acting nifedipine (Procardia)
Nitrates	Patients with anginal symptoms despite use of beta blockers or calcium channel blockers	Evidence lacking on mortality benefit
Antiplatelet agents		
Aspirin	All patients (75 to 162 mg per day), unless contraindicated	Decreases nonfatal MI, strokes, vascular deaths

Clopidogrel (Plavix)	Patients in whom aspirin is contraindicated or not tolerated	Approved for acute coronary syndrome, recent MI, stroke, peripheral arterial disease, or coronary stent placement
Lipid-lowering agents		
Ezetimibe (Zetia)	Patients who have not achieved LDL goal despite statin therapy or who are intolerant of statins	Evidence lacking on mortality benefit
Fibrates	Patients with triglycerides of 200 to 499 mg per dL (2.26 to 5.64 mmol per L) and non-HDL > 130 mg per dL (3.37 mmol per L); triglycerides ≥ 500 mg per dL (5.65 mmol per L)	Reduction to non-HDL < 100 mg per dL (2.59 mmol per L) reasonable; treat if triglycerides ≥ 500 mg per dL to prevent pancreatitis
Nicotinic acid	Same as for fibrates; triglycerides of 200 to 499 mg per dL and non-HDL > 130 mg per dL; triglycerides ≥ 500 mg per dL	Same as for fibrates; reduction to non-HDL < 100 mg per dL reasonable; treat if triglycerides ≥ 500 mg per dL to prevent pancreatitis
Statins	Patients with a baseline LDL ≤ 100 mg per dL	Initiate with lifestyle measures; reduction to LDL < 70 mg per dL (1.81 mmol per L) or high-dose statin therapy reasonable

Table .1: Pharmacotherapy for Myocardial Ischemia

1.10. Haemostasis and Thrombosis

Hemostasis is derived from a Greek word, which means stoppage of blood flow. The combination of cellular and biochemical eventsthat function together to maintain the nature of blood the liquid form within the veins and arteries thereby prevent the blood loss following injury through the formation of blood clot.[23,24]. Whenever a blood vessel is severed or ruptured, hemostasis is achieved by several mechanisms they includes vascular constriction, formation of a platelet plug, formation of a blood clot as a result of blood coagulation, and eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently. This will stop bleeding and are eventually dissolved through the fibrinolytic process. As a result of this mechanism, there is delicate balance between the production and dissolution of clot during the haemostatic process. Any disturbance in this balance may precipitate thrombosis or hemorrhage as a result of hypocoagulation or hypercoagulation respectively[25,26]. Process of hemostasis is categorized as either a primary or secondary process.

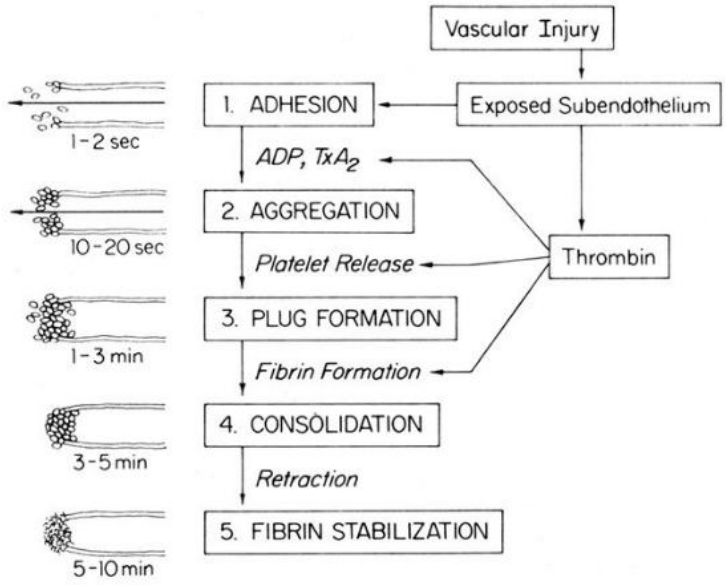


Fig 10: The Haemostatic Plug Formation

Primary hemostasis involves the response of the vascular system and platelets to vessel injury[4]. It takes place when there are injuries to small blood vessels during which the affected blood vessels contract to seal off the wound and platelets are mobilized, aggregate, and adhere to components of the subendothelium of the vasculature. Presence of various factors such as von Willebrand factor (vWF) and platelet receptors (IIb/IIIa and Ib/IX)are required for the adheasion of platelets. Further extra platelets are attracted to the site of injury by the release of platelet granular contents, such as adenosine diphosphate (ADP). The interaction of fibrinogen stabilizes the formed platelet plug.

So a defect in the function of platelet or von Willebrand's disease (vWD) may result in debilitating and sometimes fatal hemorrhage [23]. Secondary hemostasis involves the response of the coagulation system to vessel injury[26]. It is necessary to control bleeding from large wounds and is a continuation of the primary hemostatic mechanism.Whereas theplatelet plug formation is the primary hemostatic result and the outcome of secondary hemostasis is the formation of a thrombus.

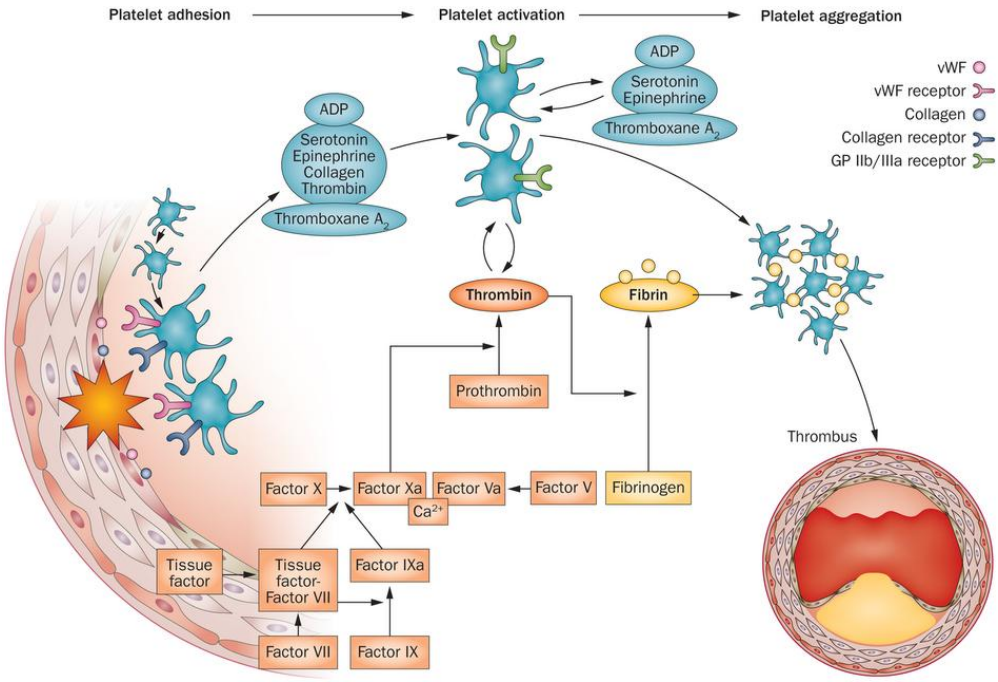


Fig. 11: Thrombus Formation

1.11. Thrombus vs. Embolus

A clot in the blood that adheres to a vascular lumen is called a *thrombus*, whereas an intravascular clot that floats in the blood is termed an *embolus*. Thus an embolus may generated as a result of a detached clot that floats in the blood. This two forms also dangerous, because, they may occlude in the blood flow route and deprive tissues of oxygen and nutrients[27].

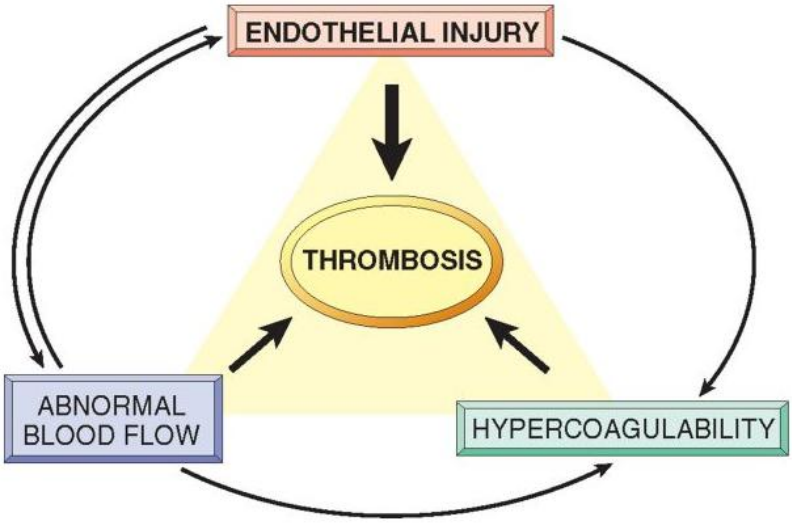


Fig. 12: Virchow's triad in thrombosis

1.12. Types of Thrombosis

There are two distinct forms of thrombosis, each of which can be presented by several subtypes.

1.13. Venous Thrombosis (Phlebothrombosis)

Venous thrombosis is triggered by the blood stasis or inappropriate activation of the series of coagulation cascade, frequently as a result of a defect in the normal hemostatic defense mechanisms 27. Majority of venous thrombi occur in the superficial or deep veins

of the leg[28]. Although these type thrombi can cause local congestion, swelling, pain, and tenderness, they rarely embolize.

Deep venous thrombosis (**DVT**) in the larger leg veins—at or above the knee (e.g., popliteal, femoral, and iliac veins)—is more serious because such thrombi more often embolize to the lungs and may produce pulmonary infarction. At the same scenario they can cause local pain and edema, venous obstructions from DVTs can be rapidly offset by collateral channels. Approximately 50% of affected individuals DVTs are asymptomatic. They are recognized only in retrospect after embolization[21].

The common predisposing factors are bed rest and immobilization (because they reduce the milking action of the leg muscles, resulting in reduced venous return), and congestive cardiac failure (also a cause of impaired venous return). Trauma, surgery, and burns not only immobilize a person but are also associated with vascular disturbence, procoagulant release from injured tissues, increased hepatic synthesis of factors of coagulation, and altered t-PA production. Tumor-associated inflammation and coagulation factors (tissue factor, factor VIII) and procoagulants (e.g., mucin) released from tumor cells and they all contribute to the increased risk of thromboembolism in disseminated cancers, so-called migratory thrombophlebitis or Trousseau syndrome[29, 30]. Regardless of the specific clinical setting, advanced age also increases the risk of DVT.

Different classes of deep vein thrombosis are,

- Portal vein thrombosis
- Renal vein thrombosis
- Jugular vein thrombosis
- Cerebral venous sinus thrombosis
- Paget schroetter disease (obstruction of an upper extremity vein by the thrombus)
- Budd chiari syndrome **(**blockage of the hepatic vein or the inferior venacava)

1.14. Arterial and Cardiac Thrombosis

Atherosclerosis is one of the major cause of arterial thromboses, because it is associated with loss of endothelial integrity and with abnormal vascular flow (see Fig.12: A). Myocardial infarction can predispose to cardiac mural thrombi by causing dyskinetic myocardial contraction as well as damage to the nearest endocardium (see Fig. 12:B), and rheumatic heart disease may engender atrial mural thrombi. Besides local obstructive consequences, cardiac and aortic mural thrombi can also peripherally embolizes. Although any of the tissues can be affected, the brain, kidneys, and spleen are particularly likely targets because of their rich blood supply[21].

Arterial thrombosis most often formed in medium-sized vessels rendered thrombogenic by surface lesions on endothelial cells caused by atherosclerosis and this usually consists of a platelet-rich clot.

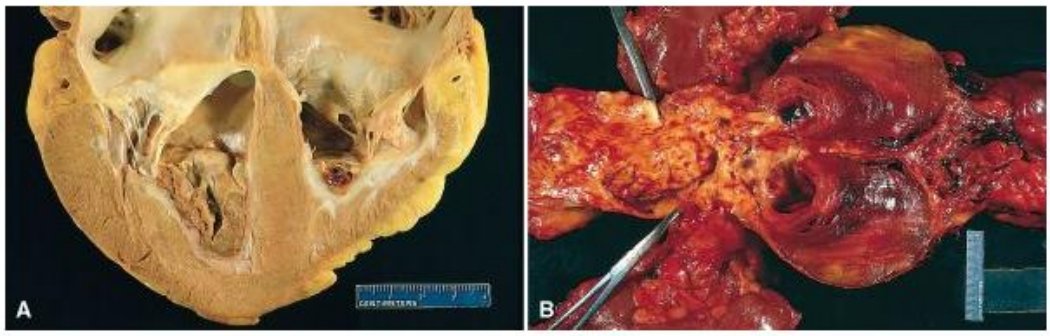


Fig. 13: A) Thrombus in the left and right ventricular apices, overlying white fibrou scar. B) Laminated thrombus in a dilated abdominal aortic aneurysm.

1.15. Stroke

A stroke is the rapid decline of brain function due to a disturbance in the supply of blood to the brain. This can be due to ischemia, thrombus, embolus (a lodged particle) or

haemorrhage (a bleeding). In thrombotic stroke, a thrombus (a blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower. Thrombotic stroke can be divided into two categories :

- Large vessel disease
- Small vessel disease

The former affects vessels such as the internal carotids, vertebral and the circle of willis. The later can affect the smaller vessels such as the branches of the circle of willis.

1.16. Myocardial Infraction

Myocardial infraction is caused by an infract (death of tissue due to ischemia), often due to the obstruction of the coronary artery by a thrombus. Myocardial infraction can quickly become fatal if emergency medical treatment is not received promptly. If diagnosed within 12 hour (hr) of the initial episode (attack) then thrombolytic therapy is initiated.

1.17. Diagnosis of Myocardial Infarction

The initial step in making the diagnosis of a blood clot is obtaining a patient history. The blood clot a does not cause any problem but the location of the blood clot and its effect on blood flow that causes symptoms and signs.

If a blood clot or thrombus is a consideration, the history may expand to explore risk factors or situations that might put the patient at risk for forming a clot. Venous blood clots often develop slowly with a gradual onset of swelling, pain and discolouration. Symptoms of a venous thrombus will often progress over hours. Arterial thrombi occurs as an acute event. Tissues need oxygen immediately and the loss of blood supply creates a situation in which symptoms begin immediately.

There may be symptoms that precede the acute artery blockage that may be the warnings signs of the potential future complete occlusion of the blood vessel.

- Patients with an acute heart attack (myocardial infraction) may experience angina in the days and weeks prior to the heart attack.
- Patients with peripheral artery disease may have pain with walking (claudication) and a transient ischemia attack, mini stroke may precede a stroke.

Physical examination can assist in providing additional information that may increase the suspicion for a blood clot.

1.18. Thrombolytic Agents[31]

Generation	Fibrin specific	Nonfibrin specific
First	……. …….	Streptokinase Urokinase
Second	Recombinant tissue plasminogen activator (t-PA)	Prourokinase (scu PA)
Third	Alteplase	APSAC
	Tenecteplase (TNK-tPA)	
	Reteplase	
	Monteplase	
	Lanoteplase	
	Pamiteplase	
	Staphylokinase	
	Desmoteplase	
	(Bat-PA) Chimeric thrombolytics	

Table .2: List of Thrombolytic Agent

1.19. Thrombolytic Therapy in Acute Myocardial Infarction

Streptokinase and front-loaded alteplase regimens are commonly used for thrombolysis in acute myocardial infarction (AMI)[32]. The success of the therapy is limited by reocclusion because reperfusion is not always achieves3[3,34]. The balance between prothrombotic and thrombolytic processes can be shifted toward thrombolysis by administration of plasminogen activators; there by procoagulant effects of such drugs have been reported[.35–43] .These side effects are important because of a procoagulant state in acute coronary syndromes[43-48].

Thrombin activation was markedly increased in patients with AMI and thrombolytic therapy associated with failure to open the occluded coronary artery and with a high reocclusion rat. No direct data on plasmin activation are available in this situation, but a recent clinical study measuring indirect plasmin markers proved the activation of the kallikrein– contact-phase system after streptokinase in patients with AMI[45].

1.20. Adverse Effects of Thrombolytic Therapy

There are number of side effect which may occur following the administration of thrombolytic agents. With the exceptions of urticaria (due to an allergic reaction) and the effects of reperfusion, all side effects may be lumped into one category, that is hemorrhage. Whether it is intracranial, conjunctival, internal, etc., it is bleeding that is the major side effect. Handle these patients very gently to avoid these problems.

1.21. Herbal Remedies for Cardiovascular Diseases

Ever since the birth of mankind there has been a relationship between life, disease and the nature. Primitive men started studying diseases and its counter remed.

Ayurveda is a comprehensive natural health care system that has been originated in India more than 5000 years ago[49]. Herbal medicines are being used by about 80% of the world

population primarily in the developing countries for their primary health care needs. They have stood the test of time for their safety, efficacy, cultural acceptability and rare side effects. The active chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions plant based medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials[50]. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy), vincristin, vinblastin, pancitaxel..etc[51].

Plants are rich in a variety of compounds. Many of them are secondary metabolites and include aromatic substences, most of which are phenols or oxygen substituted phenol derivatives such as tannins. Many of these compounds have antioxidant properties. Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of various drugs or as models for pharmacologically active compounds[52].

1.22. Cardioprotective Herbs

More than 2000 plants have been mentioned in the Traditional (Herbal/Alternative) systems of medicine and some of these are providing comprehensive relief to the people suffering from cardiovascular diseases, specially “hyperlipidemia” and “ischemic heart disease”. WHO reports that around eighty percent(80%) of the global population still

relies on botanical drugs and several herbal medicines have advanced to clinical use in modern times[53].

1.23. Herbal Cardioprotective Compounds

Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exertes free radical scavenging activity. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer also[54-55].

The naturally occurring methoxyphenol apocynin has been found to inhibit NADPH oxidase upon activation by enzyme peroxidases (e.g. soybean peroxidase, myeloperoxidase) or ROS under mild reaction conditions. Upon catalyzed activation of peroxidase , the apocynin oxidation products act to block the assembly and activation of NADPH oxidase. Although the mechanism of inhibition of NADPH oxidase remains largely unknown, apocynin's high effectiveness and low toxicity makes it a promising lead compound in the development of new therapeutic substense for cardiovascular diseases[56].

Examples of herbal agents shows cardio vascular activity: Garlic preparations are taken by many patients because of their anti-lipid and anti-platelet effects, significant factors in the prevention of thrombus formation[57-59].

The allicin derivative of garlic root has been shown to enhance fibrinolytic activity and inhibit the platelet aggregation in patients having coronary artery disease[60-62], either via a dose-dependent alteration in the production of arachidonic acid metabolites (i.e. inhibition of thromboxane formation in platelets[61,63,64] or by altering physiochemical properties (i.e; the ADP receptor) of the platelet membrane[65,66].

Consumption of polyphenols in the diet has been shown to reduce the likelihood of morbidity and mortality from coronary artery disease. One way in which polyphenols are thought to act is through the inhibition of lipid peroxidation of low density lipoprotein (LDL) [67]. Dietary supplements rich in polyphenols, such as black and green tea[68], olive oil [69], red wine, and licorice root extract[70], are associated with an increased resistance of plasma LDL oxidation[71].

1.24. Natural Thrombolytic Remedies

A regular intake of blood thinning foods against the cause of blood clots (anti- thrombotic diet) offers a convenient and effective method of prevention of acute thrombotic events. Foods and blood thinning medications have the following way to acting to prevent heart attack or stroke.

Anti clumbingof blood

Prevent blood clot formation

Clot dissolving ability

Natural blood thinning foods include strawberries, tomatoes, mulberries, onions, carrots, common thyme and rosemary etc.

1.25. Nigella Sativa: Pharmacognostic Description

Nigella sativa also known as Black seed is an herbaceous annual plant and it is indigenous to the Mediterranean region. Black cumin is One of the miracle herbs plant that has been considered as prophetic medicine is Habbatus sauda (Nigella Sativa). Seeds of Nigella sativa L. (Ranunculaceae) or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of many of diseases and conditions that include asthma, diarrhea and dyslipidaemia. N. sativa seeds are used to prepare highly prized nutritive oil. Although on the world scale Nigella seed oil does not really have a significant economic market share, yet, it nevertheless constitutes a niche market whose

size is constantly growing due to its alleged pharmacological Plant extracts and essential oil showed a broad range of pharmacological activities[72].

1.26. Taxonomic classification of Nigella sativa

Kingdom	: Plantae.
Subkingdom	: Tracheobionata that is, vascular plant.
Supervision	: Spermatophyte.
Order	: Ranunculales.
Family	: Ranunculaceae-Butter cup family.
Genera	: Nigella.
Species	: sativa.

Fig 14: Nigella Sativa Flower and Seed.

1.27. Synonyms and Habitat[73]

Black cumin, Black Caraway, Fennel Flower, Nutmeg Flower, Black seed, Roman Coriander, Damascena, Devil in-the-bush, Wild Onion Seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia.

1.28. Morphology of the Plant[73]

N. sativa is an annual flowering plant grows at 20-90 cm tall, with finely divided leaves; the flowers having5-10 petals and white, yellow, pink, pale blue or pale purple color. The fruit is a large and inflated capsule consists of 3-7 united follicles, each containing several seeds. Seeds are small dicotyledonous, trigonus, angular, tubercular, black externally and white inside, odor slightly aromatic and taste bitter.

1. 29. Nigella sativa Seed

Seeds are small dicolyledonous, trigonus, angular, regulose-tubercular, 2-3.5 × 1-2 mm, white inside covered by black external coating. Transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by papillose cuticle and filled with dark brown contents. 2-4 layers of thick walled tangentially elongated parenchymatous cells have been seen just near to the epidermis, followed by reddish brown pigmented layer composed of thick walled, rectangular elongated cells[74].

1.30. Active Constituents

Thymoquinone, the active constituent of Nigella sativa seeds, is pharmacologically active quinone, which prevents oxidative injury in various in vitro and in vivo studies in rats[75,76] and also has been suggested that thymoquinone may quench oxidant radicals and prevents membrane lipid peroxidation in tissues [77]

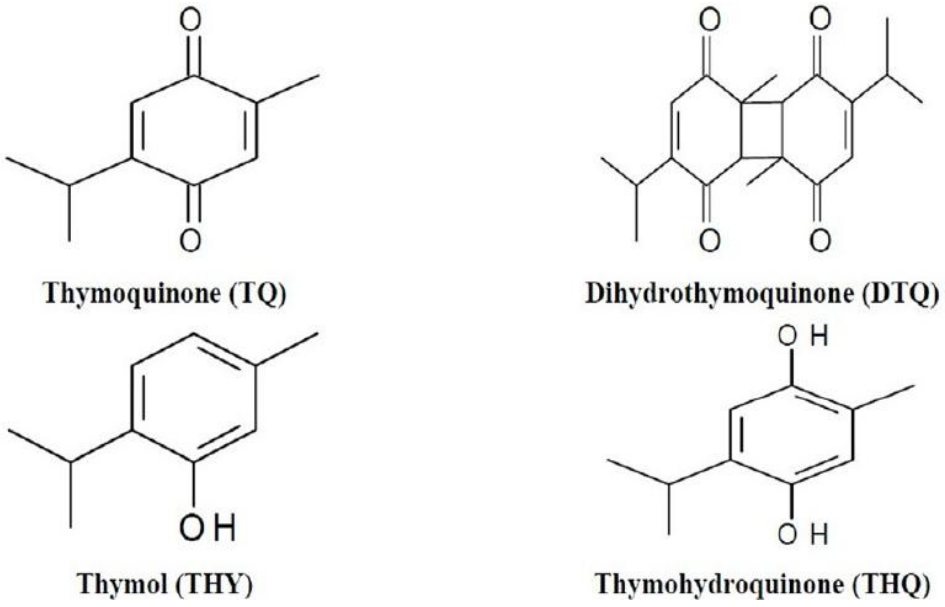


Fig. 15: Different chemical components of Nigella Sativa

1.31. Pharmacological Effects for Potential Healthcare

Traditionally its seeds and oil are used in several diseases. The seeds are considered as bitter, pungent, aromatic, appetizer, stimulant, diuretic, emmenagogue, galactogogue, anthelmintic, acride, thermogenic, carminative, deodorant, digestive, constipating, febrifuge, exppectorent, purgative, antihypertensive, bronchodilator, gastroprotective, hepatoprotective, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, analgesics and anti-inflammatory, spasmolytic, renal protective and antioxidant properties.[78]

1.32. Hordeum Vulgare: Botanical Description[79]

Barley is a member of the grass family Triticeae. Wheat and rye also fall into this grass family. In total, there are thirty-one barley species. Of the thirty-one, three-fourths are perennial grasses, blooming every summer and dying back in the winter. Although there

are so many types of barley, scientists hypothesize that they all originated from one ancestor plant, the Hordeum spontaneum.

Fig 16: Barley plant and grains.

1.33. Taxonomic Classification of Hordeum vulgare

Kingdom	: Plantae - Plants
Subkingdom	: Tracheobionta - Vascular plants
Superdivision	: Spermatophyta - Seed plants
Division	: Magnoliophyta - Flowering plants
Class	: Liliopsida – Monocotyledons
Subclass	: Commelinidae
Order	: Cyperales
Family	: Poaceae - Grass family
Genus	: Hordeum – barley
Species	: H. vulgare

Barley is an annual grass that strands 60-120 cm tall. Barley has two types of root systems, seminal and adventitious. The stems are erect and made up of hollow, cylindrical

internodes separated by the nodes, which bear the leaves.A mature barley plant consists of lateral stem and 2-5 branch stems, called tillers[80].

Barley leaves are linear 5-15 mm wide and are produced alternate sides of the stem. The leaf structure consists of the sheath, blade auricles and ligules. The sheath surrounds the stm compleately. The ligules and auricles distinguishes the barley from other cereals as they are smooth, envelop the stem and can be pigmented with anthocyanins[81].

Barley cell walls encapsulate starch granules embedded in a protein matrix. With thin cell walls and loose packing of endosperm, the large mealy grains allow a rapid water up-take and uniform distribution of water and enzymes synthesised during germination. On the contrary, due to thick cell walls and tightly packed endosperms, small steely grains retard mass transfer in the endosperm. Large, plumb kernels are desired for malting. The fraction above the 2.5 mm sieve is normally used for malting and the rest is included in the feed fraction. A larger uniform grain size is desired because it enables homogenous water up-take and modification[79].

1.34. Nutritional Information

One cup (237 ml) of cooked pearled barley contains 193 calories, while the whole-grain (hulled) form contains 270 calories and contains as much protein as a cup (237 ml) of milk.

1.35. Pharmacological Effects for Potential Healthcare

N. sativa has been extensively studied for its biological activities and shown to possess wide spectrum of activities such as diuretic, antihypertensive, bronchodilator, gastroprotective, hepatoprotective, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial,analgesics and anti-inflammatory, spasmolytic, renal protective and antioxidant properties[79]

CHAPTER – II

LITERATURE REVIEW

1. **Kaur *et al* (2013)** reported that More than 2000 plants have been listed in the Traditional (Herbal/Alternative) systems of medicine and some of these are providing comprehensive relief to the people suffering from cardiovascular diseases, specially "hyperlipidemia" and "ischemic heart disease".[82]

2. **Francis *et al* (2002)** reported that In the clinical assessment of chest pain, electrocardiography is an essential adjunct to the clinical history and physical examination. The ST segment elevation associated with an evolving myocardial infarction is often readily identifiable, but a knowledge of the common "pseudo" infarct patterns is essential to avoid the unnecessary use of thrombolytic treatment.[83]

3. **Marwa A *et al* (2013)** investigated as Niella Sativa Oil supplementation attenuates lead-induced cardio toxicity by mechanisms related, at least in part, to its ability to decrease the pro inflammatory cytokines, oxidative stress and cardiac tissue damage and preserve the activity of antioxidant enzymes. *N. sativa* could serve as a true functional food and may positively affect health promotion via reducing cardiovascular risk.[84]

4. **Hosseini *et al* (2014)** found that the extracts of nigella sativa with glycyrrhiza glabra and zingiber officianalis have some protective effects against DOX-induced toxicity in cardiomyocytes with similar efficacies, but with different potencies. However, NGZ produced much higher protective effect via reducing oxidative stress and inhibiting of apoptotic induction processes. Further investigations are needed to determine the effects of NGZ on DOX chemotherapy.[85]

5. **Kaleem *et al* (2008)** indicated that oral administration of ethanolic extraction of nagella sativa seeds to streptozotocin induced diabetic rats 30 days significantly

reduces the elevated levels of blood glucose, lipids, insulin and improved the altered levels of lipid peroxidation products and antioxidant enzymes like catalase, super oxide dismutase,reduced glutathione and glutathione peroxidase in liver and kidney.[86]

6. **Kaatabi, *et al* (2012)** investigated that nigella sativa suppliments at a dose of 2g/ day for 12 weeks may improve the dyslipidemia associated with type 2diabetis patients. So nigella sativa is a potential protective natural agentagainst atherosclerosis and cardiovascular complication in the patients.[87]

7. **Abou Gabal *et al* (2007)** found that the treatment of the animals with N. sativa improved both genotoxicity and ultrastructural changes induced by the two dose levels of CCl4.[88]

8. **Amr A. Rezq (2014)** investigated The effects of Nigella Sativa Extract (NSE), Nigella Sativa Oil (NSO) and Vitamin E (Vit. E) and the combination of NSE or NSO with Vit. E on potassium bromate (KBrO3)-induced oxidative stress in male rats. According to his conclussion oral administration of NSE or NSO alone and in combination with vitamin E exhibited weight gain and feed efficiency ratio and lowered the elevated serum levels of total cholesterol, triglycerides, AST, ALT, urea nitrogen, uric acid and creatinine[89.]

9. **Ahmed Jasim Mohammad (2015)** studied the role of black seed oil and olive oil on the cholesterol and total protein and triglycerides by using twenty-five rabbit, divided into five totals: total control of outdoor graduation was drenched just water. The first treatment series: a dose of black seed oil 25% and 75% of olive oil and the second group outdoor graduation was drenched black seed oil 50% and 50% olive oil. Then third treatment group outdoor graduation was drenched black seed oil 75% and 25% of olive oil. And group fourth treatment 100% outdoor graduation was drenched black caraway seeds only. He noticed the effect of treatment after the

duration of study, that is low cholesterol in the group treated first, second and third, depending on the concentration of black seed oil and olive oil, while increases when there are black cumin oil only in the fourth set. And the level of total protein in group treatment first, second and third while dipping in the fourth treatment group outdoor graduation was drenched black caraway seeds only. Low triglycerides in group treatment first, second and third, depending on the concentration of black seed oil and olive oil, while increases when there are black cumin oil only in Group IV according to his suggestion the large term and high concentration supplementation of black seed oil reduced plasma liquid cholesterol and triglyceride while total protein increased, from these point of view , an intake of black seed oil combination with olive oil feasible therapeutic strategy for prevention and treatment of patient with hyperlipidemia and obesity.[90]

10. **Neelam Chaturvedi *et al* (2011)** suggested that natural products such as cereals are likely to form the basis of nutraceutical as its revolution represents an enormous opportunity for growth and expansion. Wheat, rice, millets, barley, oat, buckwheat ,corn ,sorghum, flaxseed psyllium, brown rice, and products are notify the most common cereal based functional foods and nutraceuticals. The nutrients in the cereals have identified prospective for reducing the risk of coronary heart disease, diabetes, tumor incidence, cancer risk, blood pressure, reduces the rate of cholesterol and fat absorption, delaying gastrointestinal emptying and providing gastrointestinal health[91].

11. **Vaclav Vetvicka *et al* (2007)** showed that with respect to natural glucans, there is a yes-or-no effect suggesting that highly purified and highly active glucans will have pleiotropic impact, whereas poorly isolated and/or less active glucans will have only mediocre biological properties[92].

12. **Mokhtar I *et al* (2006)** demonstrated that treatment of diabetic rats with barley and some of its components (chromium and amino acids) could repair liver damage and

restoring pancreatic b-cells deformation. This was manifested by the biochemical and immunoassay results and electron microscope study where the hypoglycemic and hypolipidemic action of barley may be due to its contents generally and in specific to its content of chromium and/or amino acids[93].

13. **Gul *et al* (2014)** demonstrates that HV possesses activities against all human platelet agonists used in this study except ADP, and inhibited both COX and LOX pathways of AA metabolism. It also elevated the activities of SOD and GPx. However, these activities were distributed in various fractions. Therefore, it is unlikely that a single phytocompound is responsible for all these activities. Some fractions of HV showed more potent activities than others while certain fractions were almost completely devoid of anti-inflammatory effect (aqueous). Interestingly, crude extract was unable to enhance GPx activity while n-hexane fraction which was obtained from the fractionation of crude extract, caused significant elevation of GPx activities[94].

14. Consumption of soluble fiber improves risk factors for cardiovascular diseases and diabetes mellitus. It also provides satiety value. Soluble fiber reduces plasma cholesterol concentrations, lowers postprandial plasma glucose and insulin concentrations and ameliorates insulin resistance. Most research on soluble fiber has focused on oats. Barley, another excellent soluble fiber source, has received little attention. Many forms of barley or barley extracts have not been investigated in human subjects. Thus, research is needed to assess the health effects of human consumption of barley and barley products including germinated barley foodstuff, barley co-products, and barley Nutrim. This paper describes research that uses controlled feeding of human subjects to determine the ability of barely and barley products to affect risk factors for cardiovascular disease and diabetes in normal weight and overweight adults. Moreover, the research will assess the ability of diets high in soluble fiber to aid in weight loss and maintenance of weight-reduced subjects[95].

15. **Inas E Darwish *et al* (2013)** studied that barley extract orally administered had reinforced the venlafaxine antidepressant and antioxidant effect when combined together in experimental induced chronic mild stress model in rats. Their compliment effect had explored further on brain serotonin and magnesium serum level. No significant effect on brain nitric oxide level had been proved in the current study by any of the treated regimen[96].

16. **Saurabh Arjariya *et al* (2013)** evaluated that the potential toxicity of *Terminalia catappa* (almond) in rats. Acute toxicity of the *Terminalia catappa* extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. No dose related toxicities are obsereved in his14 days investigation. In the chronic toxicity study, oral administration of the aqueous extract of *Terminalia Catappa* linn at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. There was no toxicity/ death was observed at the dose of 2000mg/kg b.w. In the chronic toxicity study, no significant treatment-related changes in the levels of hematological and biochemical parameters. It suggests that the aqueous extract of Terminalia catappa linn. does not have significant toxicity[97.]

17. **Rabiatul *et al* (2014)** reported that there is no statistically significant effect of concentration of 0.1, 0.5 and 2.0g/kg cinnamon aqueous extract (CE) on oral sub acute toxicity study on behaviour, mortality, water intake, food consumption, weight gain, internal organs weight (liver and kidney) and heamatological parameters during treatment and post-treatment periods. But a slight decreased in kidney and liver weight of rats treated with 0.5g/kg and slight decreased in liver weight of rats treated with 2.0g/kg, during post-treatment period. So these evaluation study suggested that the CE is low to moderate in toxicity and CE below 0.5 g/kg dose level is safe to be used in the efficacy study especially for diabetes treatment[98].

18. **Prasanth kumar *et al* (2014)** performed a study on acute and sub acute (28-day) oral assessment of ethanolic extract of celtis timorensis leaves in mice using OECD

425 guidelines whereas sub-acute toxicity study was carried out in rats by using OECD 407 guidelines. Mice where administered a single dose of 2000mg/ kg and 5000 mg/kg orally in acute toxicity study. Then observed individually for first four hours, then over a period of 24 hours and at least once daily for 14 days. In the case of oral sub-acute toxicity studies, EECT was given orally at doses of 250, 500, 1000 mg/kg body weight daily for 28 days to male and female rats respectively. General behavior, adverse effects and mortality were observed during the period of the study. Water intake, food consumption, relative organ weight, hematological and biochemical parameters and organ histopathological alterations are evaluated[99].

19. **Behera *et al* (2012)** examined the phyto-constituents and cardioprotective activity of *Pongamia pinnata (PP)* leaf extract (hydro-alcoholic) in experimentally induced myocardial infarction in Wistar Albino rats. Six groups of Wistar albino rats, each comprising six animals, were selected for the study. Group I served as a control, Group II rats were given isoproterenol (20 mg/100g, subcutaneously), and Group III rats were given *Pongamia pinnata* leaf extract (300 mg/kg). Groups IV, V and VI rats were given *Pongamia pinnata* leaf extract (100 mg/kg, 200 mg/kg and 300 mg/kg, respectively) and isoproterenol (20 mg/100g subcutaneously) prior to MI induction. The transaminases (aspartate transaminase and alanine transaminase), lactate dehydrogenase (LDH) and creatine phosphokinase (CK), were estimated in both the plasma and heart tissues. Troponin T activity was separately estimated for plasma.during their study it is noticed that the Isoproterenol significantly increased the activities of CK, LDH and the transaminases in plasma with a concomitant decrease in these enzymes in tissue. Pretreatment with the hydro-alcoholic leaf extract of *Pongamia pinnata* at a dose of 300 mg/kg body weight for 30 days had a significant effect on the activities of marker enzymes compared to the other groups. *Pongamia pinnata* pretreatment may offer protection in experimental cardiotoxicity induced by isoproterenol[100].

20. **Abi Beaulah *et al* (2014)** reported that the Methanolic extract of *Croton sparciflorus* showed significant cardioprotective effect by lowering the serum levels of various biochemical parameters like CPK, LDH and transaminases in the selected model. The results obtained in the present work are in turn with histopathological examinations of heart tissue sections and are comparable with the standard cardioprotective drug. The results also suggests that the biologically active phytoconstituents such as flavonoids, glycosides, alkaloids present in the methanolic extract of plant which is confirmed from the qualitative analysis may be responsible for the significant cardioprotective activity[101].

21. **PakutharivuThangarajan *et al* (2015)** demonstrated the effect of Pithecellobium dulce fruit peel in isoproterenol (ISO) induced myocardial infarction (MI) in adult male Wistar rats. Myocardial infarction was induced by intraperitoneal injection of isoproterenol (ISO) 2 mg/kg in experimental rats. Aqueous and ethanolic extract of Pithecellobium dulce fruit peel was administered intraperitoneally at a dose of 200 mg/kg for a period of 14 days. On 14th day the rats were induced with ISO. Serum lipid profile, liver marker enzymes such as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT), cardiac marker enzymes such as creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were measured in the experimental rats along with histopathological studies of heart tissues. ISO-induced rats showed a significant increase in the activities of marker enzymes such as SGOT, SGPT, CPK and LDH. Pretreatment with aqueous and ethanolic extract of Pithecellobium dulce fruit peel, positively altered the activities of marker enzymes and the biochemical parameters in ISO-induced rats. Thus the study showed that Pithecellobium dulce possess cardioprotective effect in ISO induced MI in rats[102].

22. **Alam Firoj *et al* (2014)** repoted that T. belerica possess potential to ameliorate the myocardial damage induced by isoproterenol in rat. . Myocardial damage was induced by subcutaneous administration of isoprenaline (85 mg/kg) for two

consecutive days. . Male wistar rats were administered Terminalia belerica (150 and 300mg/kg/p.o.) for 28 days and metoprolol (meto, 10 mg/kg) for 28 days orally in their respective groups. A change in biomarkers levels reflects the influence of treatment. The creatine kinase (CK) activities were fallen in serum and elevated in heart tissue of animals treated with low and high doses of T.B as well as Metoprolol compared to ISO control. The enzymes-lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum activity were significantly reduced inserum with both and high doses of T.belerica while no change was noted in heart with both doses compared to ISO control. Hence it is concluded that T. belerica possess potential to ameliorate the myocardial damage induced by isoproterenol in rat[103].

23. **E. Sathyapriya *et al* (2012)** studied the in vitro antiplatelet aggregation and thrombolytic activity of cheenalinga chendhuram (CLC) drug from mineral origine, which was showed effective at the dose of 300µg/ml and 75µg/ml respectively.so they conclude therefore, that CLC is an effective drug in the treatment of cardiovascular diseases and cerebrovascular accidents[104].

24. **Bhaargavi V *et al* (2016)** investigated the thrombolytic potential of natural plants and their products. The invitro clot lysis activity was studied by taking various proportion of herbal mixtures and compare with streptokinase as positive control and isotonic normal saline as negative control. The aqueous extracts of Punica granatum, Zingiber officinale and Phyllanthus emblica on an average were able to dissolve 37.42%, 30.03% and 34.31% of the clot respectively[105].

25. **A. Elumalai *et al* (2012**) investigated with crude extract of Bogainvilla glara carried out to evaluate its possible thrombolytic activity. The crude methanolic extract was found have significant thrombolytic activity at a dose of 800 micro gram/ ml with maximum effect comparable with the streptokinase as positive control and water as negative control[106].

26. **Mubarak yusuf Ibrahim *et al* (2015)** worked out an in-vitro thrombolytic model was used to check the clot lysis effect of the crude extract of B. pilosa, streptokinase was used as positive control and sterile water used as negative control. In this study model, methanoli extract of B.pilosa showed significant clot lysis activity with 46.20±2.274 % when compared to positive control (82.60± 2.45 %) and negative control (11.29 ± 0.677 5). Base on these study results they suggested that the thrombolytic activity of B.pilosa, could be considered as very promising and beneficia for the traditional medicine[107].

CHAPTER - III

AIM AND OBJECTIVE OF THE STUDY

Cardiovascular diseases (CVDs) are group of disorders of the heart and blood vessels and include coronary heart disease (heart attack), cerebrovascular disease, rheumatic heart disease and other conditions. Four out of five cardiovascular disease deaths are due to heart attacks and strokes.

Cardiovascular Diseases are responsible for over 17.3 million deaths per year and are the leading causes of death in the world. Deaths due to heart attacks, strokes and other types of CVDs as a proportion of total cardiovascular deaths for males and females.

AIM:

The primary aim of this study is to evaluate the cardiovascular assessment of dual herbal combination.

To met the primary aim the following objectives are considered

- To assess the acute and sub-acute toxicity study of the dual herbal combination
- To determine the cardioprotective activity by isoproterenol induced myocardial infarction in experimental animal model
- To perform the thrombolytic activity by in-vitro screening method.

CHAPTER-IV

PLAN OF WORK

Proposed plan of work was carried out in the following stages:

Phase I

- Literature survey

Phase II

- Collection of herbal ingredients and raw materials.

Phase III

- Toxicological assessment
- Acute toxicology (OECD 425)
- Sub-acute toxicology (OECD 407)

Phase IV

- Pharmacological evaluation
- Cardioprotective evaluation
- In-vitro thrombolytic activity

Phase V

- Compilation of data and conclusion

CHAPTER – V

MATERIALS AND METHODS

5.1. Herbal Components

Name of the herbal ingredients	Name of the manufacturer
Black seed oil	Nigella's herbal healthcare, Calicut, Kerala. Drug. lic. no.: 06/25D/2011
Barley powder	Ashtavaidya herbal pharma, Ettumanoor, Kerala. Mfg. lic. no.:FSSAI11312005001123

Table 3: Name and manufacturer of the herbal ingredients

The herbal mixture of nigella oil and barley water was prepared and stabilized with tween 80

5.2. Chemicals

Name of the chemicals	Name of the supplier
Isoproterenol hydrochloride	Micro labs limited, API division, Banglore-560105, Karnataka, India.
Chloroform	Nice chemicals, Kochi,Kerala
Formaline	Merk Millipore, India
Normal saline	Baxter india, Haryana
Tween 80	S D fine chemicals ltd, Mumbai
Di sodium hydrogen orthophosphate (mono and dibasic)	S D fine chemicals ltd, Mumbai

Table 4: Name and supplier of the chemicals used

5.3. List of Instruments

Name of instruments	Name of the supplier
Rota-rod Apparatus	INCO, instruments and chemicals
Cooks Pole Climbing Apparatus	MKM,Chennai
Photo -Actometer	INCO, instruments and chemicals
Heated plate analgesiometer	INCO, instruments and chemicals

. **Table 5: Name and supplier of the instruments**

5.4. Experimental Animals

Male and female albino wistar rats (150- 200 g) used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages under standard environmental conditions (12H dark /12H light cycles; temp., 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water ad libitum. The animals were acclimatized to the environment for two weeks prior to experiment use. The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethical committee.

5.5. Approval of Protocol

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of RVS College Of Pharmaceutical Sciences,Sulur, Coimbatore constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment

and Forests, Government of India (Reg. No. 1012/PO/c/CPCSEA). Ethical guidelines were strictly followed during all the experiments.

METHODS

5.6. Toxicological Evaluation

5.6. A. Acute Toxicity Studies[99]

Oral acute toxicity studies of NSO+BW (Black Cumine Oil + Barley Water) was carried out in female rat by using Organization for Economic Co-operation and Development (OECD) guideline 425. The rats were deprived food for 3 hour before oral administration of a single dose of the test samples. Doses of 5 ml of nigella sativa oil followed by 5 ml of barley water were given using oral gavage needle to rats of group 1 and group 2 respectively. All the test sample given animals were observed for general behavioral changes; toxicity symptoms and mortality after treatment for the first four (crucial) hours. Then over a period of 24 hours, there after daily for 14 days.

5.7. B. Sub- Acute Toxicity Studies

Sub- acute 28-day repeated dose oral toxicity study was carried out according to OECD 407 guidelines[2]. Both sexes of rats (150-200g) were divided into four different groups with 10 animals (5 male and 5 female each).

Group I : Saline 10ml/kg

Group II : Low dose (0.5ml NSO +1 ml BW)

Group III : Medium dose (1 ml NSO + 2 ml BW)

Group IV : High dose (2 ml NSO + 4 ml BW)

All the groups were observed two time in a day for mortality and morbidity till the completion of experiment. The clinical signs and time of onset, duration of these symptoms, if any were recorded. The amount of food and water intake was noticed on every day and data were expressed as 7 days cumulative value. Body weights of the treatment groups were recorded once before the start of dosing, once weekly during the period and finally on the day of sacrifice. At the end of the experiment (on 29th day), blood samples were collected from overnight fasted rats by retro- orbital puncture method into EDTA coated or uncoated sample collecting tubes for hematological and biochemical analysis[99].

5.6. C. Hematological Parameters

The EDTA mixed blood was used for the analysis of hematological parameters such as total hemoglobin (Hb), packed cell volume (PCV), WBC count, RBC count, differential count, MCV, MCH, MCHC, RDW, platelet count and MPV etc.

5.6. D. Biochemical Parameters

The serum was separated from the uncoated (EDTA) blood and the serum biochemical parameters including total cholesterol, creatinine, bilirubin total, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (Tg), HDL, LHL, VLDL, CHO/HDL ratio were analyzed by using various biochemical laboratory analyzing methods .

5.6. E. Histopathology

On the 29th day, all the animals were euthanized after blood collection for gross pathological examinations of all major organs such as liver, kidney, brain and heart were collected from the animals for histopathogical study. The collected organs were weighed and preserved in 10 % neutral buffered formaline, trimmed and microne sized tissue sections were stained with appropriate staining agent (hematoxylin and eosin) for histopathological examination.

5.7. Pharmacological Studies

5.7. A. Assessment of Cardioprotective Activity[109]

5.6. A. I. Allocation of Groups And Experimental Protocol

The rats were randomly divided into five groups with six rats in each group.

Group I : Normal animals received saline 10ml/kg body weight with standard feed and water to allow ad libitum throughout the experimental period.

Group II : The rats were orally fed normal saline once daily for 30 days and in addition, received isoproterenol (85mg/kg body weight) on the 29 and 30 day at an interval of 24 h.

Group III : Rats were pretreated with verapamil (5μmol/kg body weight, intera vein) for a of 14th day and 30th days only and in addition, received isoproterenol 85 mg/kg body weight on the 29 and 30 day at an interval of 24h.

Group IV : Rats were pre-treated with NSO + BW for a period of 30 days and in addition, received isoproterenol 85mg/kg bw on the 29 and 30day at an interval of 24 h.

5.7. A. II. Induction of Myocardial Infarction

At the end of experimental period, all the animals, except the normal untreated rats that served as the control group, were administered isoproterenol (ISO) 85 mg/kg, interaperitoneal injection for two consecutive days on the 31 and 32 day at an interval of 24

h. to induce myocardial injury After 48 hours rats were anaesthetized with anaesthetic ether, then sacrificed and the hearts were harvested for biochemical and histological studies.

5.7. A. III. Collection of Blood and Heart Tissues

After treatment of 32nd day, all the animals were sacrificed by cervical dislocation by mild chloroform anaesthesia and the fasting blood sample of each group were collected separately into sterilized blood collecting tubes, and allowed to coagulate for 30 min. at 37 ^{0}C. The clear serum obtained after centrifugation was used for the estimation of biochemical parameters like lactate dehydrogenase (LDH), creatine kinase (CK-MB), serum aspartate amino transferase (AST), and alanine aminotransferase (ALT).

The heart was excised immediately and immersed in physiological saline. It was suspended in 10%) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces.

5.7 A. IV. Histological Examinations

The heart was excised immediately and washed with ice-cold saline; then fixed in 10% buffered formalin; 10% stored buffered formalin were embedded in paraffin; 5µm thick sections were cut and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes with the help of a veterinary pathologist.

5.7. B. In-vitro Thrombolytic Activity[110]

5.7. B. I. Effect of NSO+BW on Clot Lysis

Blood sample (500µl) was distributed in pre weighed sterile micro centrifuge tubes and incubated at 37 ^{0}C for 90min for clot formation. After clot formation, the serum was completely aspirated without disturbing the clot and the tubes were again weighed to determine the clot weight

Clot weight = Weight of the tube containing clot – Weight of the empty tube.

To each micro centrifuge tube containing the pre weighed clot, 100µl of NSO+BW was added separately. As, a positive control 100µl of streptokinase (SK) and as a negative non thrombolytic control 100µl of distilled water added separately to the different control tubes. All the tubes are then incubated at 37° C for 90 mins and observed for clot lysis. After incubation the released fluid was removed and the tubes were weighed again. The difference in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown.

% of clot lysis = (weight of released clot/ clot weight)* 100.

5.8. Statistical Analysis

All the results are expressed as mean ± SEM (standard error mean). Data obtained was analyzed by using one way ANOVA followed by dunnett's test and $p< 0.05$ was considered as statistically significant.

CHAPTER – VI

RESULTS AND DISCUSSION

6.1. Acute Toxicity Study

In acute toxicity study, oral administration of the NSO+BW at (0.5+1ml/kg and 1.5+4ml/kg) lower dose and higher dose did not produce any deaths and clinical signs of toxicity in experimental rats. Both the test doses does not show mortality and clinical signs of toxicity, therefore LD50 value of NSO+BW was found to be greater than higher dose.

LD 50 determination is usually an initial step in the determination and evaluation of toxic characteristics of a substance in the preliminary drug screening. This initial assessment of toxic manifestation is one of the primary screening experiments performed with all lead compounds.

Data from the acute toxicity study may provide; basic information on the mode of toxic action of substance; help in dose determination in animals; help arrive at a dose of new compound; and most importantly help to determine LD 50 values that provide many indices of potential types of drug activity.

NSO+BW at a dose of 1.5+4ml/kg had no changes in Eye, Skin, Fur, Mucosal membrane, respiratory, Circulatory, Autonomic, CNS, Motor and Behavioral pattern were noted.
The observed results show no signs of tremor, convulsion, salivation, diarrhea, lethargy and sleep etc (Table: 4)

Sl no	Parameters	Observations
1	Reactivity	Easy/normal
2	Handling	Did not resist, very easy to handle
3	Palpebral closure	Normal (eyes are open)
4	Lacrimation	No lacrimation
5	Salivation	No salivation
6	Piloerection	None/normal
7	Hair coat	Normal
8	Bite marks	None
9	Nail status	Normal
10	Rearing activity	Normal
11	Clonic involuntary movement	None
12	Tonic involuntary movement	None
13	Gait	Normal
14	Movements	Normal
15	Arousal	Normal (keeps guard up and engages in exploratory activity)
16	Stereotype behaviour (preening, squeaking, shaking head and other repetitive behavior	None
17	Abnormal behaviour (squirming, running backwards, labored movements, squealing)	None

Table 6: Effect of NSO+BW on Autonomic Observations

6.2. Sub-Acute Toxicity Study

In the repeated dose 28 days oral sub-acute toxicity study, there is no significant toxicity signs and mortality observed in the both sexes of rats treated at doses 0.5+1 ml, 1+2 ml and 2+4 ml of body weight of NSO+BW.

No treatment related changes in body weight were observed between the initial and final body weight of rats treated with NSO+BW and control (Table: 7). A similar lack of toxicity was noticed in the case of food and water consumption (Table: 5 and 6) during the period. There were no significant differences between groups of animals treated with NSO+BW and control in organ weight (Table: 8).

6.2.1. Effect on Feed Intake

Treatment group	Sex	Feed Intake (gms)			
		Week 1	Week 2	Week 3	Week 4
control	Male	46.23±0.90	64.56±1.32	49.75±1.70	36.32±2.43
	Female	44.34±0.295	65.47±0.495	42.85±1.885	34.22±0.74
LD	Male	77.24±1.65	76.21±1.65	61.34±2.24	41.73±3.63
	Female	79.01±1.09	74.11±1.55	51.32±4.22	37.2±1.455
MD	Male	67.43±2.04	70.20±1.95	63.21±1.90	50.38±1.67
	Female	65.71±2.2	62.15±0.935	45.38±0.56	44.9±1.935

HD	Male	65.26±2.32	69.52±2.46	53.79±3.06	54.48±3.26
	Female	67.73±2.375	64.71±1.285	45.64±2.17	43.43±1.005

Values are expressed as mean ±SEM, n = 5 females and 5 males

Table 7: Effect of combination of NSO+BW on Feed Intake in Rats-Sub-Acute Toxicity Study

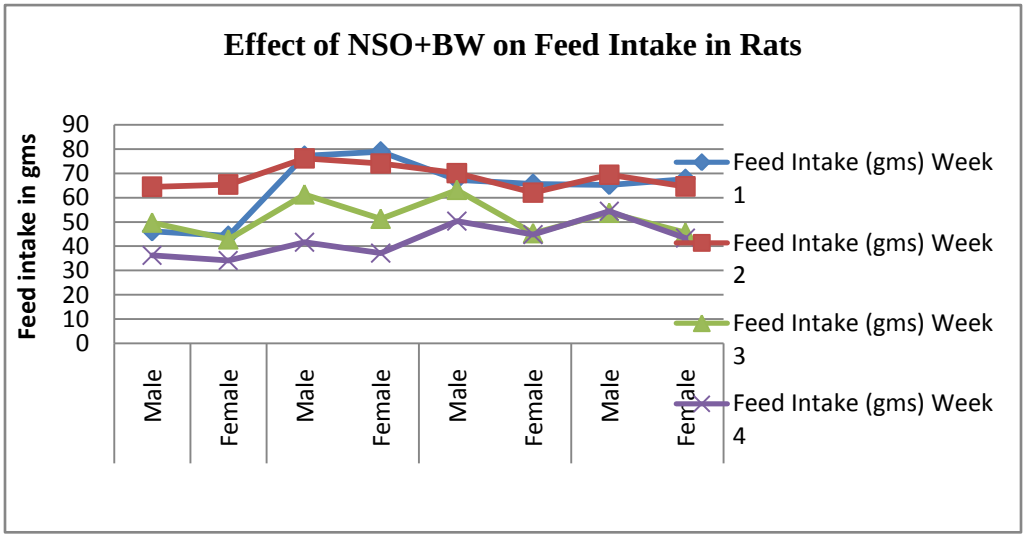


Fig 17: Graphical representation Effect of NSO+BW on feed intake in control and experimental rats (sub acute toxicity studies).

6.2.2. Effect on Water Intake

Treatment group	Water Intake(ml)				
	Sex	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	Male	67.61±1.76	63.82±1.41	66.79±2.82	60.43±1.52
	Female	63.00±1.425	64.90±2.34	62.48±1.77	46.19±0.825
LD	Male	76.55±2.13	75.64±1.14	71.32±1.42	55.92±0.85
	Female	75.29±2.45	74.47±1.39	52.64±1.045	53.48±1.025
MD	Male	69.20±2.14	70.25±3.50	43.76±3.32	52.43±2.39
	Female	65.84±0.385	60.91±0.475	45.12±0.76	46.13±0.79
HD	Male	65.21±1.02	72.30±1.31	46.61±0.92	50.50±1.38
	Female	66.37±0.725	65.65±0.965	45.82±0.54	45.42±1.435

Values are expressed as mean ±SEM, n = 5 females and 5 males

Table 8: Effect of combination of NSO+BW on Water Intake in Rats-Sub-Acute Toxicity Study

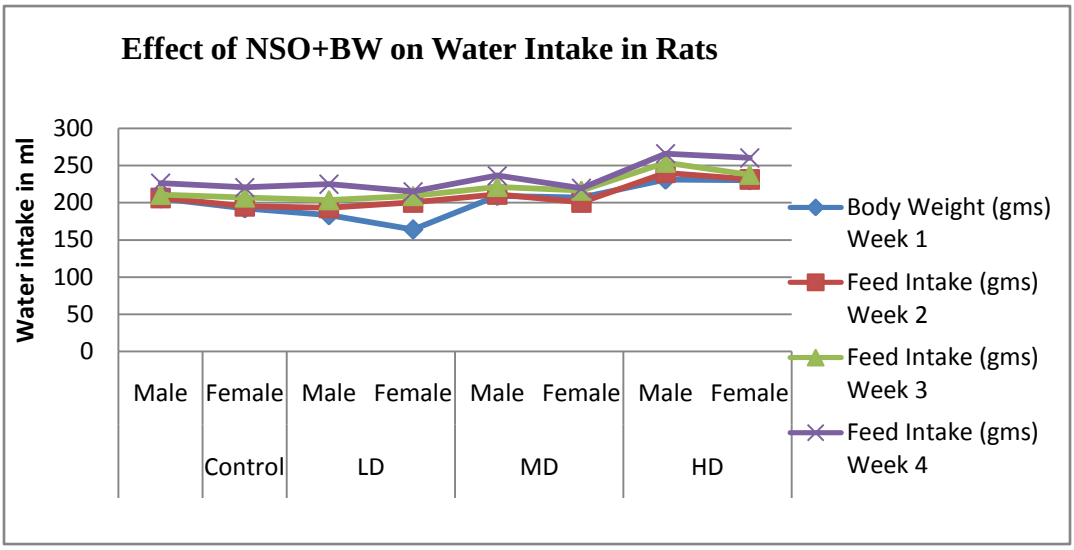


Fig 18: Graphical representation Effect of NSO+BW on water intake in control and experimental rats (sub acute toxicity studies).

6.2.3. Effect on Body Weight

Treatment group	Sex	Body Weight (gms)			
		Week 1	**Week 2**	**Week 3**	**Week 4**
Normal	Male	205.5±4.57	206.5±3.32	210.6±4.25	226.4±5.55
	Female	192.25±4.5	195±3.725	207±4.405	220.5±6.34
LD	Male	183±4.56	193±3.31	203.4±4.35	225±5.09
	Female	163.8±3.325	200.2±4.09	209±6.335	215±8.07
MD	Male	209±5.45	211±5.78	221.3±5.94	236.4±7.25

	Female	206.75±1.65	200.25±2.015	216.25±2.52	219.5±1.84
HD	Male	231±5.66	240±4.32	254±6.45	266±4.45
	Female	230±5.4	231.25±4.26	237.75±2.92	260.25±3.52

Values are expressed as mean ±SEM, n = 5 females and 5 males

Table 9: Effect of combination of NSO+BW on Body Weight Gain in Rats-Sub-Acute Toxicity Study

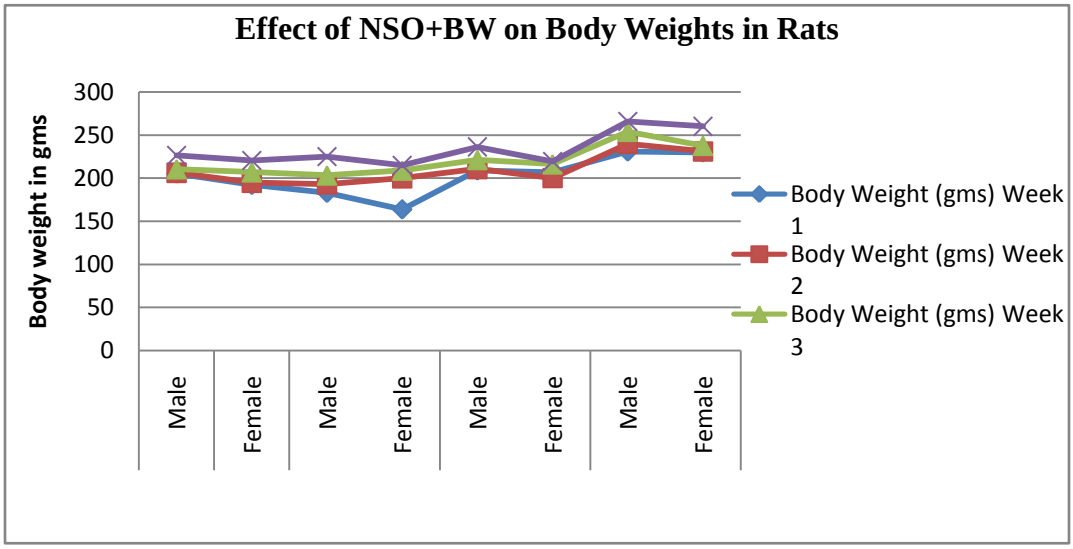


Fig 19: Graphical representation Effect of NSO+BW on body weights in control and experimental rats (sub acute toxicity studies).

6.2.4. Effect on Relative Organ Weight

Organ weight (gms)	Sex	Treatment group			
		Control	**LD**	**MD**	**HD**
Heart	Male	0.71±0.03	0.67±0.03	0.75±0.04	0.69±0.02
	Female	0.65±0.02	0.74±0.05	0.75±0.05	0.70±0.075
Liver	Male	4.89±0.20	5.85±0.31	5.82±0.45	5.31±0.25
	Female	4.66±0.35	5.87±0.705	5.11±0.315	5.04±0.115
Kidney	Male	0.98±0.12	1.15±0.06	1.08±0.05	1.10±0.05
	Female	1.02±0.05	1.18±0.08	1.15±0.05	1.09±0.05
Brain	Male	1.45±0.05	1.55±0.05	1.65±0.06	1.67±0.05
	Female	1.53±0.02	1.61±0.09	1.55±0.075	1.52±0.05

Values are expressed as mean ±SEM, n = 5 females and 5 males

Table 10: Effect of combination of NSO+BW on organ weight in Rats-Sub-Acute Toxicity Study

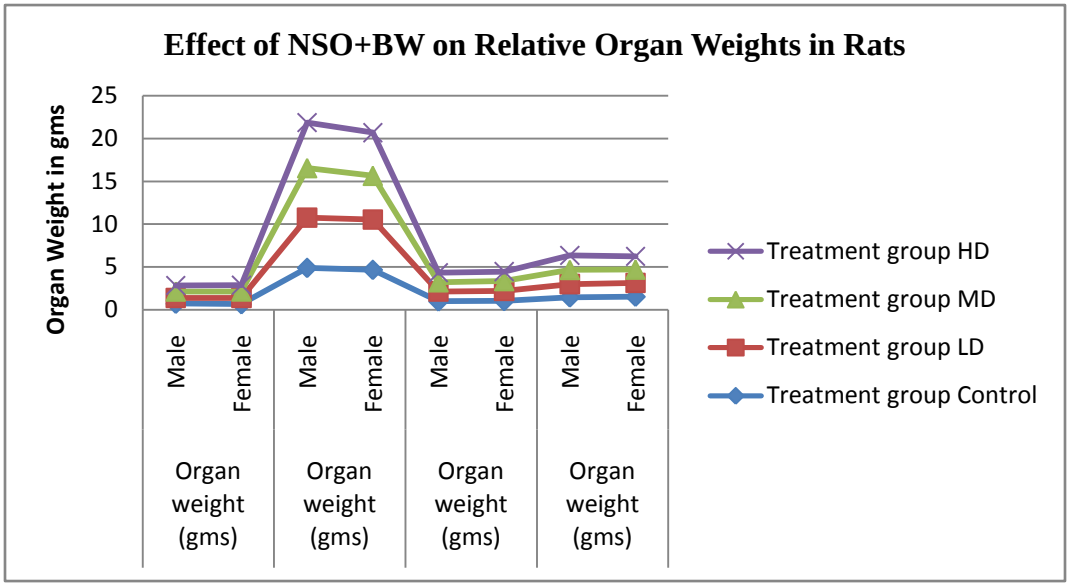


Fig 20: Graphical representation Effect of NSO+BW on relative organ weight in control and experimental rats (sub acute toxicity studies).

6.2. 5. Effect on Hematological Profile

The hematological data of treated and control groups were summarized (table: 5) and concluded that the evaluated parmeters such as total RBC, total WBC,HB, PCV, MCV, MCH, MCHC, RDW, MPV, Polymorph, Lymphocyte, Eosiniphils, Monocytes and platelet count etc are within normal range in both control and treated groups during the experimental period.

Hematological parameters	**Sex**	**Treatment group**			
		Control	**LD**	**MD**	**HD**
Hemoglobin (g/dl)	Male	14.74±0.67	14.92±0.58	14.75±0.64	15.01±0.42
	Female	15.68±0.21	16.38±0.70	16.9±0.28	16.45±0.63
PCV (%)	Male	46.43±1.86	53.31±1.75	53.42±1.44	50.50±1.90
	Female	47.36±2.03	52.28±2.14	54.92±2.28	52.6±3.08
Total WBC Count ($x10^3$/µl)	Male	8.84±0.68	9.54±0.70	9.18±0.62	9.46±0.41
	Female	10.48±0.15	10.80±0.43	9.695±0.57	10.85±0.38
Total RBC Count ($x10^6$/µl)	Male	4.72±0.63	4.91±0.25	4.96±0.23	5.1±0.17
	Female	5.14±0.59	5.98±0.36	6.74±0.24	6.9±0.41
Polymorphs (%)	Male	9.24±1.12	8.12±1.67	8.64±0.14	9.02±0.088
	Female	9.98±1.09	11.00±0.20	10.00±0.11	9.625±0.084
Lymphocytes (%)	Male	46.00±3.31	39.00±3.42	42.00±2.37	44.00±1.33
	Female	58.00±4.64	59.01±2.06	54.00±4.31	49.62±1.25
Monocytes (%)	Male	5.2±0.27	5.0±0.28	4.7±0.19	4.2±0.11
	Female	2.92±0.69	4.1±0.55	3.75±0.71	2.65±0.57
Eosinophils (%)	Male	2.72±0.28	2.40±0.34	2.45±0.51	2.61±0.66
	Female	2.52±0.20	2.50±0.27	3.17±0.19	3.75±.0.42

MCV	Male	56.21±3.46	55.32±3.48	55.43±3.35	63.01±3.46
	Female	71.90±2.94	73.87±0.30	74.32±2.20	73.77±4.61
MCH	Male	20.21±1.07	20.15±0.68	21.8±0.09	21.04±0.78
	Female	25.31±3.53	26.42±0.50	25.2±1.28	26.05±1.92
MCHC	Male	33.07±0.27	32.15±1.13	31.24±1.56	32.19±1.64
	Female	30.68±0.23	31.17±0.87	30.95±0.85	30.25±1.51
RDW	Male	12.30±2.67	12.14±3.36	11.76±1.07	13.46±1.10
	Female	15.68±2.45	14.9±2.37	17.62±0.37	16.05±1.14
Platelet count ($x10^3$/µl)	Male	710.34±30.01	819.32±26.24	892.28±29.14	901.71±20.56
	Female	734.23±31.22	725±27.78	749.25±31.26	768±25.71
MPV	Male	8.6±1.16	9.2±1.10	8.81±0.89	9.4±0.68
	Female	6.9±0.14	7.44±.019	7.65±0.26	6.00±0.37

Values are expressed as mean ±SEM, n = 5 females and 5 males

Table 11: Effect of combination of NSO+BW on Hematological Parameters in Rats-Sub-Acute Toxicity Study

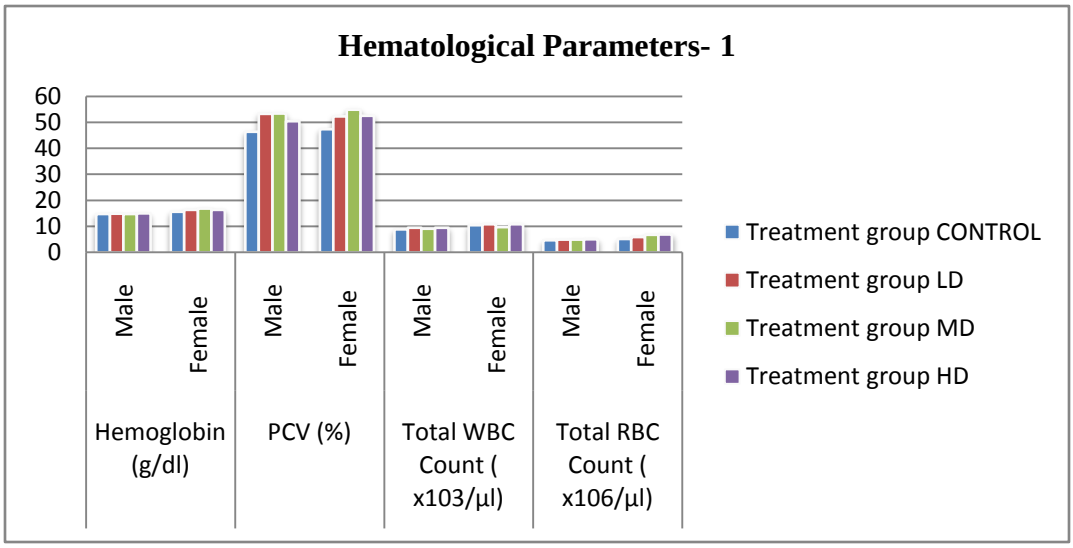


Fig 21: Graphical representation Effect of NSO+BW on hematological parameters in control and experimental rats (sub acute toxicity studies).

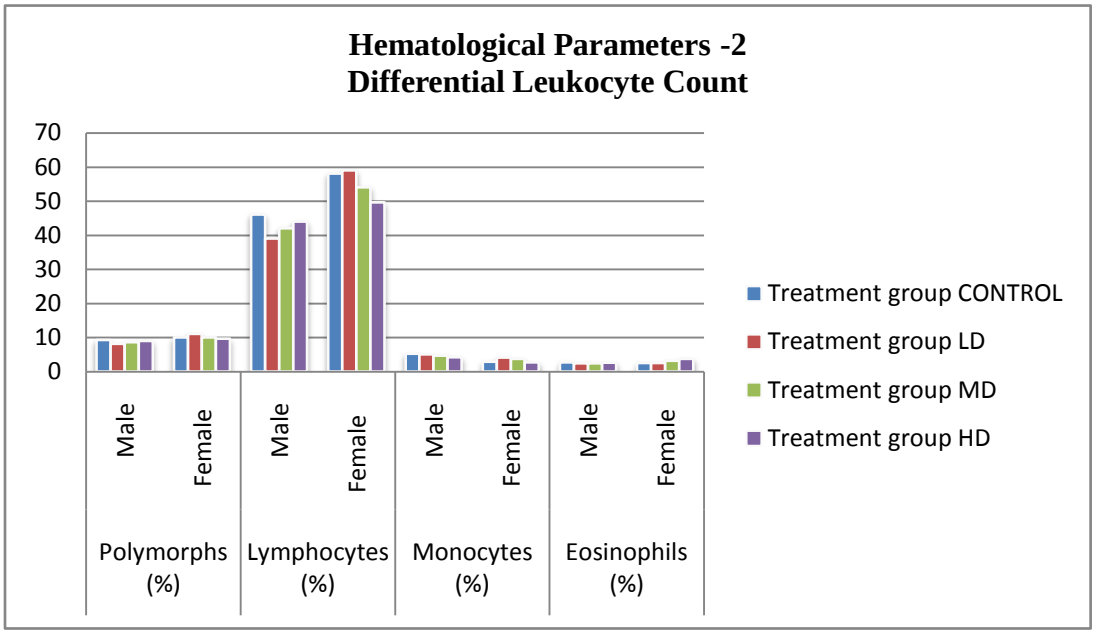


Fig 22: Graphical representation Effect of NSO+BW on hematological parameters in control and experimental rats (sub acute toxicity studies).

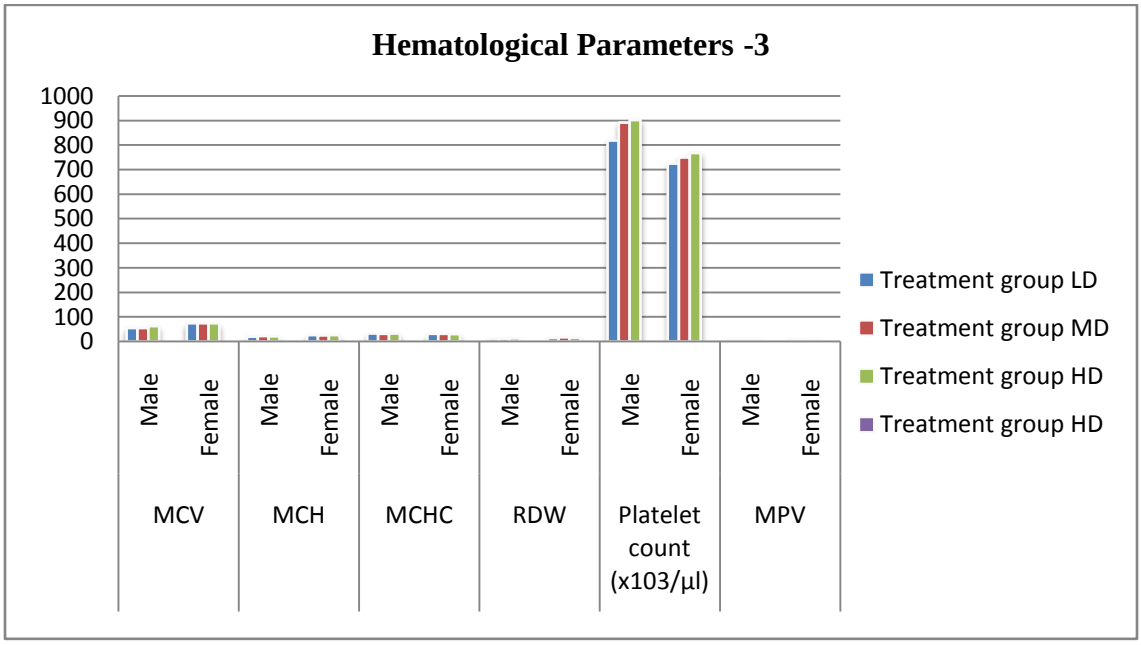


Fig 23: Graphical representation Effect of NSO+BW on hematological parameters in control and experimental rats (sub acute toxicity studies).

6.2. 6 .Effect on Biochemical Profile

The information in the biochemical parameters of the control group and treated groups were presented in table:6. In this sub acute oral toxicity study didn't show any significant changes in the biochemical parameters of the animal such as Bilirubin Total, Total Protein, SGOT(AST), SGPT(ALP), Creatinine and Lipid Profile including total cholesterol, triglycerides, HDL, LDL, VLDL, CHOL/HDL ratio.

Biochemical parameteres	**SEX**	**Treatment group**			
		Control	**LD**	**MD**	**HD**
SGOT (U/L)	Male	135.22±4.66	156.51±1.21	150.43±3.20	171.22±3.03
	Female	121.97±3.52	152.76±1.38	154.7±4.43	187.55±4.17
SGPT (U/L)	Male	40.12±2.28	45.21±1.88	47.38±1.42	43.04±1.26
	Female	41.02±3.05	42.1±0.98	44.23±0.05	42.63±0.04
Total Protein (g/dl)	Male	7.40±0.15	7.24±0.17	6.98±0.52	7.30±0.21
	Female	7.21±0.32	7.34±0.28	7.53±0.13	7.92±0.32
Creatinine (mg/dl)	Male	0.82±0.14	0.81±0.03	0.77±0.075	0.74±0.04
	Female	0.89±0.06	0.78±0.05	0.84±0.045	0.80±0.054
Bilirubin Total (mg/dl)	Male	0.83±`0.20	0.79±0.18	0.82±0.15	0.80±0.12
	Female	0.86±0.14	0.88±0.175	0.92±0.05	1.07±0.07
Total cholesterol (mg/dl)	Male	83.42±4.75	86.00±3.24	84.00±1.89	85±1.55
	Female	80.02±3.59	83.95±2.05	94.9±1.78	83.84±1.65
Triglycerides (mg/dl)	Male	89.69±8.13	164.45±7.18	169.71±8.12	160.08±5.13
	Female	141.55±10.89	160.88±15.36	155.44±3.21	158.04±1.07
HDL (mg/dl)	Male	14.2±0.88	21.63±1.36	20.15±1.52	34.31±1.09
	Female	26.47±0.45	29.98±1.68	41.3±2.24	33.37±2.47
LDL (mg/dl)	Male	20.90±1.58	26.27±2.64	30.4±1.91	23.53±2.06
	Female	21.61±1.41	16.88±1.35	16.63±1.72	14.12±0.25
VLDL (mg/dl)	Male	17.6±1.11	2.47±1.06	25.41±0.98	25.01±1.26
	Female	25.2±	34.04±1.68	29.55±2.24	31.18±1.49

CHOL/HDL Ratio	Male	2.32±2.35	2.09±1.25	2.87±1.68	2.25±1.08
	Female	1.64±0.135	2.34±1.65	2.16±1.75	2.14±0.97

Values are expressed as mean ±SEM, n = 5 females and 5 male

Table 12: Effect of combination of NSO+BW on Biochemical Parameters in Rats-Sub-Acute Toxicity Study

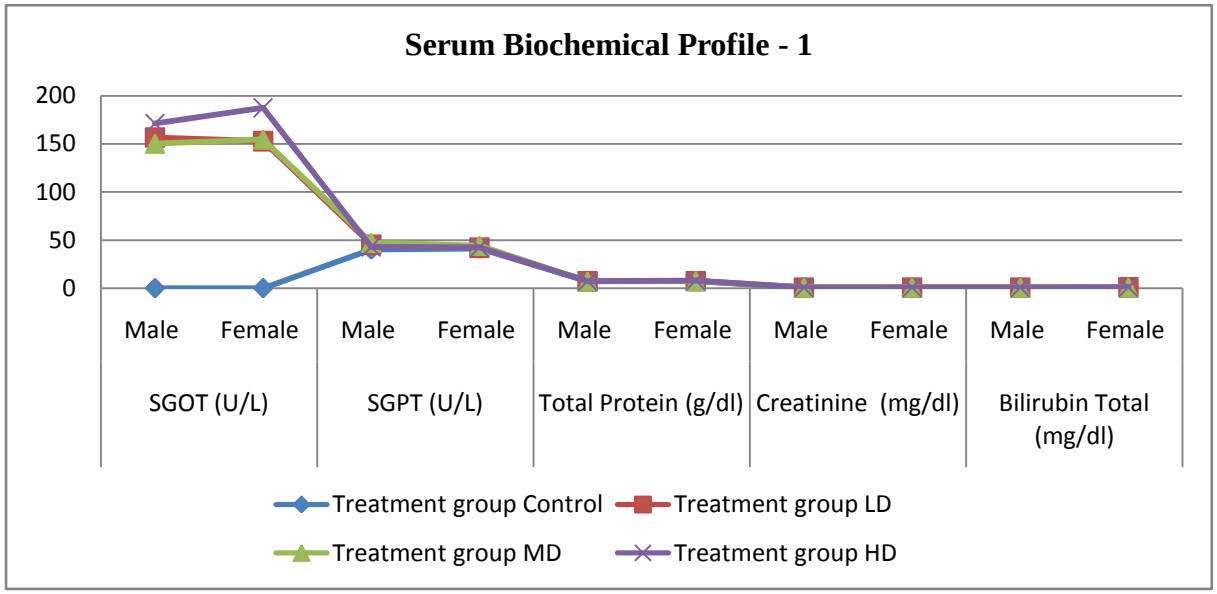


Fig 24: Graphical representation Effect of NSO+BW on serum biochemical profile in control and experimental rats (sub acute toxicity studies).

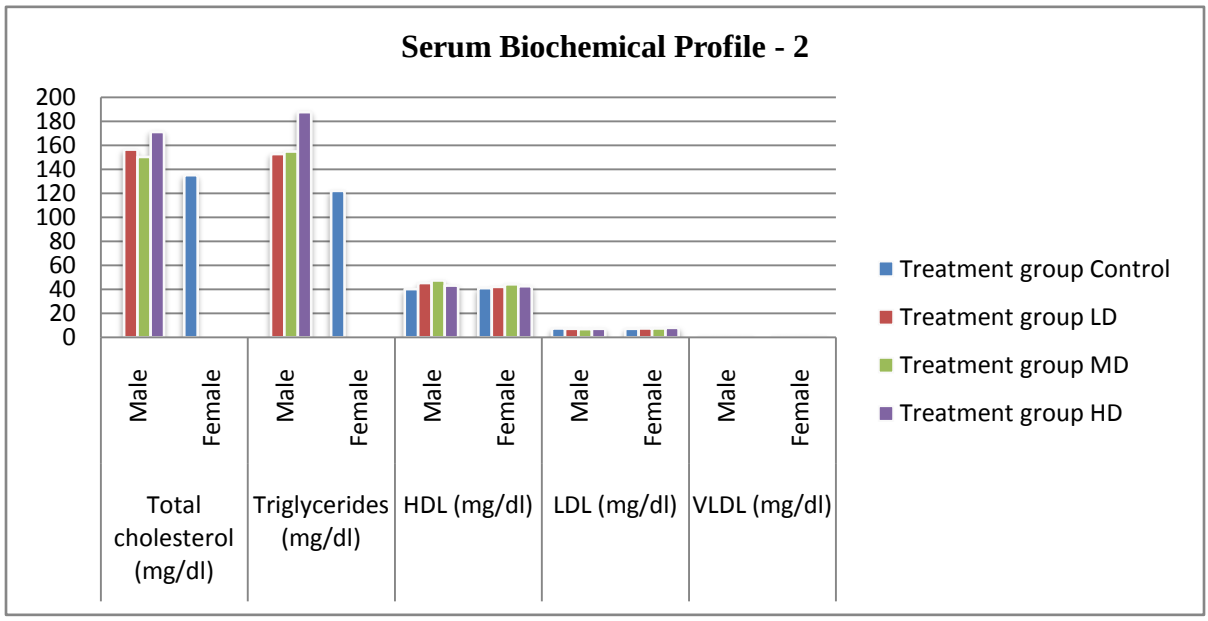


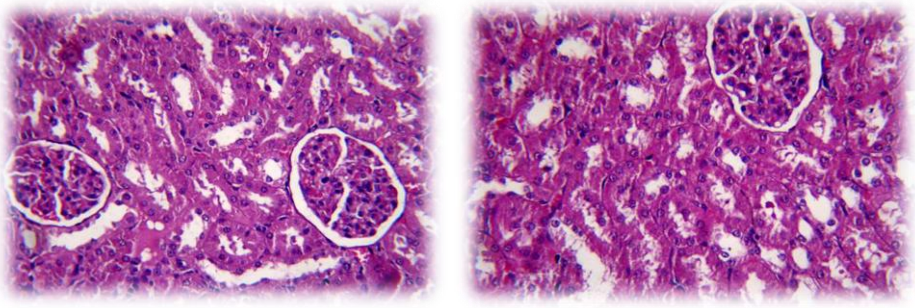
Fig 25: Graphical representation Effect of NSO+BW on serum lipid profile in control and experimental rats (sub acute toxicity studies).

.

6.2. C. Histopathological study

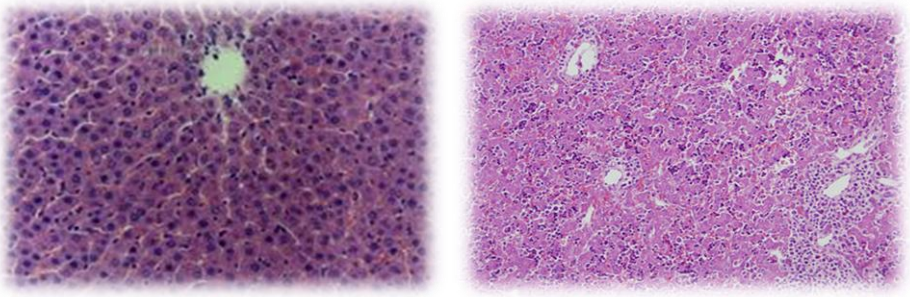
Fig.26-29 showed the histopathological changes in the kidney, liver, heart and brain of control and experimental rats after 28-day sub-acute toxicity study along with the findings for fourteen days recovery period. Fig. 26 illustrated normal architecture of kidney of the control rats as well as NSO+BW treated group. Normal structural features suggesting the preserved renal integrity of the sub-acute treatment groups.

The liver, heart, kidney and brain exihibits the normal architecture indicating the absence of any treatment related toxicity.



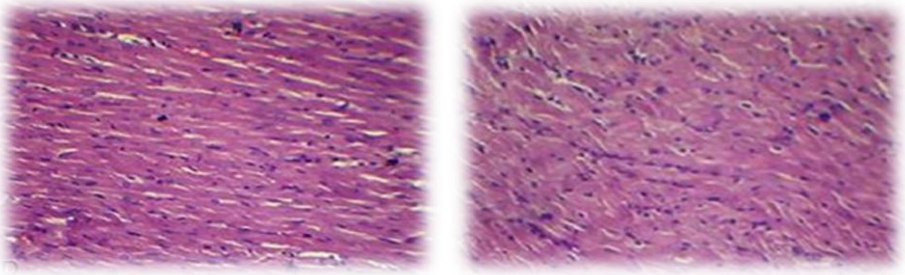
A B

Fig 26: Histological sections of kidney from the control and maximum dose A-section of kidney treated with normal saline, B-section of kidney treated with NSO+BW (2+4 ml kg^{-1})



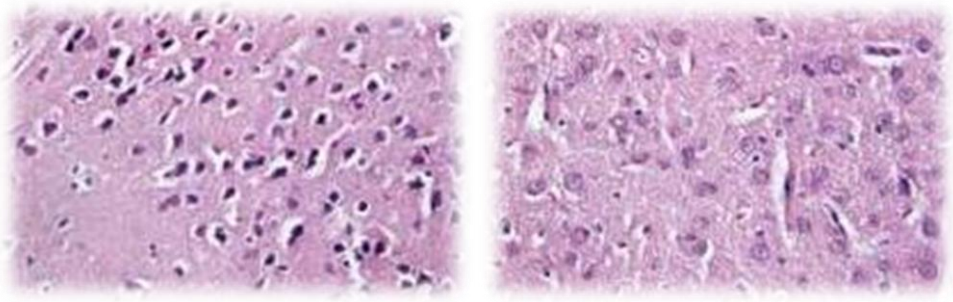
A B

Fig 27: Histological sections of liver from the control and maximum dose A-section of liver treated with normal saline, B-section of liver treated With NSO+BW (2+4 ml kg^{-1})



A B

Fig 28: Histological sections of heart from the control and maximum dose A-section of heart treated with normal saline, B-section of heart treated with NSO+BW (2+4 ml kg^{-1})



A B

Fig 29: Histological sections of brain from the control and maximum dose A-section of brain treated with normal saline, B-section of brain treated With NSO+BW (2+4 ml kg^{-1})

6.3. Cardiac Protective Activity

Parameters	Treatment Group			
	Normal	**Control (ISOonly)**	**ISO+ Propranolol**	**ISO+(NSO+BW)**
CK-MB (U/L)	123.4±3.62	174.25±5.39	134.25±4.31	130.75±4.50
LDH (U/L)	739.75±5.65	1881.75±13.22	942.47±10.15	1025.12±9.32
SGOT (U/L)	135.22±4.66	165.47±5.10	126.27±5.38	154.025±4.005
SGPT (U/L)	87.4±4.84	151.1±5.61	111.4±3.66	74.8±4.33

Table 13: Effect of NSO+BW on the level of serum cardiac markers and in normal and isoproterenol induced MI in rats.

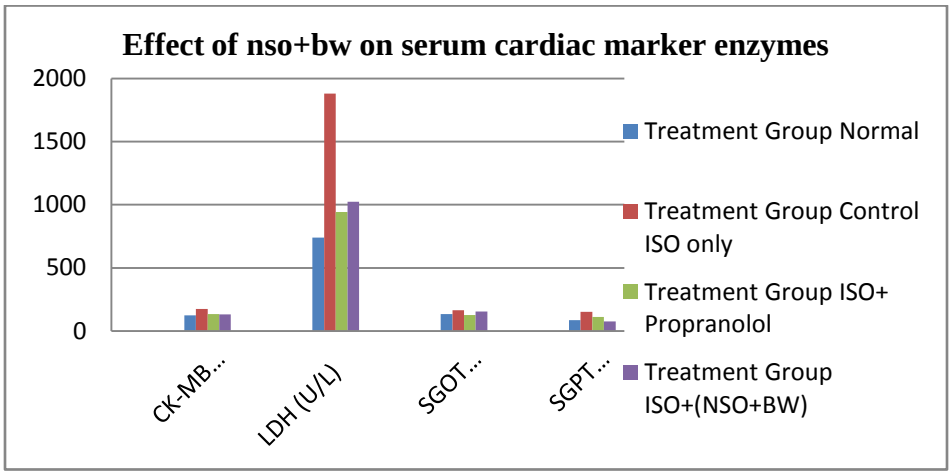


Fig 30: Graphical representation of Effect of NSO+BW on myocardial marker enzymes in ISO- induced myocardial infarction.

6.3. A. Effect on serum Creatininekinase (CK)

Rats treated with ISO (85mg/kg/,IP,) for two consecutive days had serum CK level of (174.25±5.39 U/L) measured. This was significantly higher ($P<0.05$) when compared to serum levels of CK in normal control rats (123.4±3.62 U/L) and standard ie; propranolol(134.25±4.31). ISO rats treated with NSO+BW (1+2 ml) for 30 days had serum level CK of (130.75±4.50 U/L) measured. This was significantly lower ($P<0.05$) when compared to CK levels in Iso control rats (123.4±3.62 U/L) (Table:11).

6.3. B. Effect on serum Aspartate transaminase (AST)

Rats treated with ISO (85mg/kg/,IP,) for two consecutive days had serum AST level of (165.47±5.10 U/L) measured. This was significantly higher ($P<0.05$) when compared to serum levels of AST in normal control rats (135.22±4.66 U/L) and standard ie; propranolol(126.27±5.38). ISO rats treated with NSO+BW once daily(1+2 ml) for 30 days had

serum level AST of (154.025±4.005 U/L) measured. This was significantly lower($P<0.05$) when compared to AST levels in ISO control rats (165.47±5.10 U/L) (Table:11)

6.3. C. Effect on serum Alanine transaminase (ALT)

Rats treated with ISO (85mg/kg/,IP,) for two consecutive days had serum ALT level of (151.1±5.61U/L) measured. This was significantly higher ($P<0.05$) when compared to serum levels of ALT in normal control rats (87.4±4.84U/L) and standard ie; propranolol (111.4±3.66). ISO rats treated (1+2 ml p.o, once daily) for 30 days had serum vlevel ALT of (74.8±4.33U/L) measured. This was significantly lower($P<0.05$) when compared to ALT levels in Iso control rats (151.1±5.61U/L) (Table:11).

6.3. D. Effect on Serum Lactate dehydrogenase (LDH)

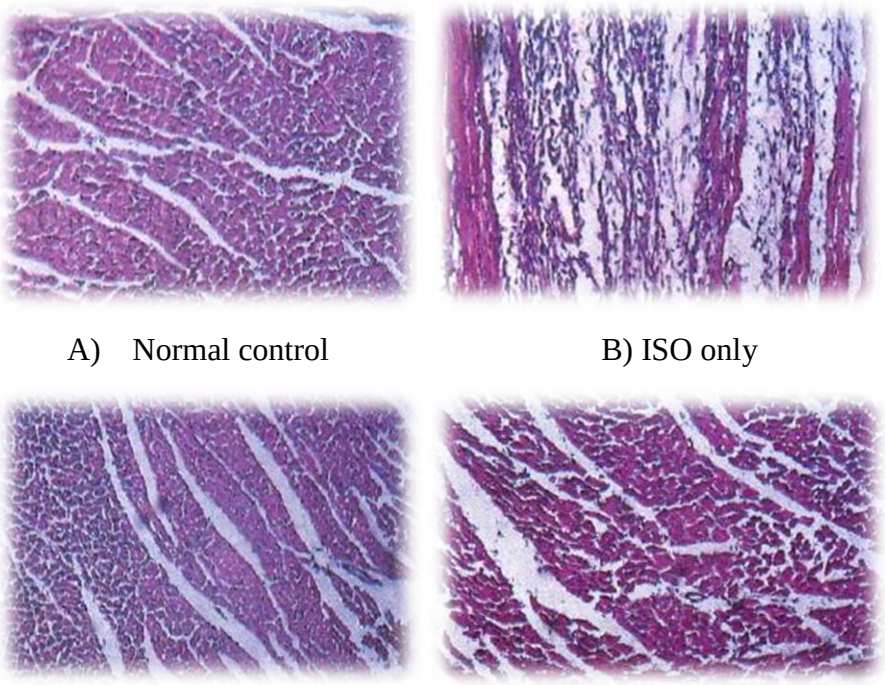
Rats treated with ISO (85mg/kg/,IP,) for two consecutive days had serum LDH level of (1881.75±13.22 U/L) measured. This was significantly higher ($P<0.05$) when compared to serum levels of LDH in normal control rats (739.75±5.65 U/L) and standard ie; propranolol (942.47±10.15). ISO rats treated with NSO+BW (1+2 ml, p.o, once daily) for 30 days had serum level LDH of (1025.12±9.32U/L) measured. This was significantly lower ($P<0.05$) when compared to LDH levels in ISO control rats (1881.75±13.22 U/L) (Table:11).

6.3. E. Histopathology of Cardioprotective Effect of NSO+BW

Histopathological examination of the normal control rat heart showed the regular histological arrangements with clear striations of myocardial fibers without any cellular alterations because of degradation of necrosis(fig 26-A) . The rats in the group 2 (ISO only) showed the abnormal histological arrangements including several congestions,

subendocardial necrosis and abundant hyperplasia along with increased edematous intramuscular space (fig 26-B). The histology of

heart of group 3 treated with standard drug ie; propranolol maintain the normal architecture with minute alteration in congestion and necrosis. The same pattern of arrangements are observed in the rat heart treated with NSO+BW of (1+2ml kg^{-1}) (fig 26- C&D.).



A) Normal control B) ISO only

C) Treated with std drug D) Treated with NSO+BW

Fig 31: Histopathological section of heart after a treatment period.

6.4. In-vitro Thrombolytic Activity

The positive control (streptokinase) after 90 min of incubation at 37 ^{0}C showed 91.00% clot lysis with p< 0.0002. the negative control (distilled water) showed only 14.67% clot lysis. Percentage clot lysis obtained after the treatment with NSO+BW (Table 12).was noticed a n impressing result when compared with positive control (significant, p<0.002).

Treatment group	Percentage clot lysis (Mean ± S.D)
Streptokinase	91.00±0.55
Distilled water	14.67±0.92
NSO+BW	84.79±1.25

The values expressed as mean ± SD, p<0.0002and p<0.002 by t-test method

Table 14: Result of thrombolytic assay

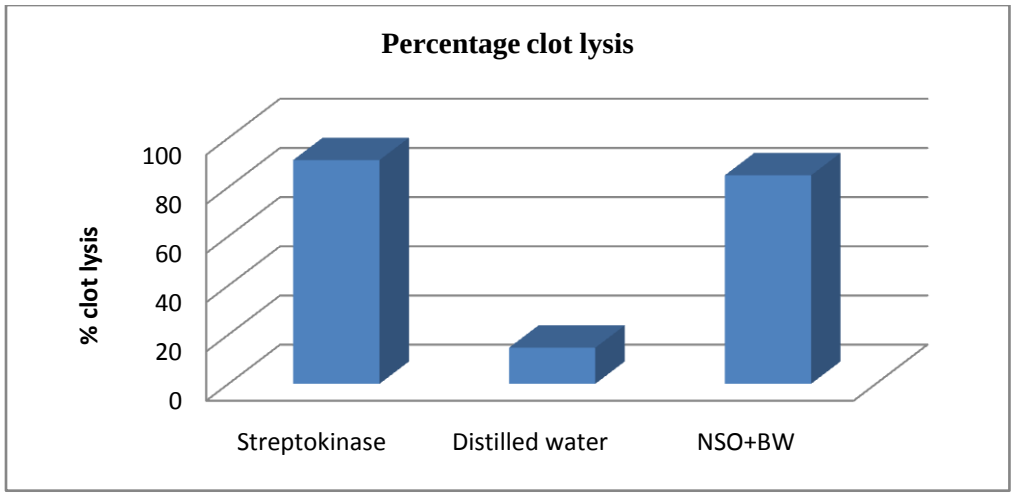


Fig 32: Graphical representation of in-vitro thrombolytic activity of NSO+BW

DISCUSSION

Acute toxicity and Sub-acute toxicity[97-99]

In the developing countries, herbal products are prepared from various medicinal plants have become famous in health care and obtained from natural sources. It is well known that the herbal medicines commonly contain more than one plant or active constituents and their therapeutic efficacy is not provided by a single group of compounds. Some of these compounds act synergistically to modify the bioavailability and efficacy of the active constituent[2]. The bioactive compounds from the medicinal plant are concluded to be safe without knowing the possible health care benefits and thus commonly used as self medication. However, there is a defect on toxicological data of these compounds. So acute toxicity study is required to identify the range of doses and probable clinical signs evoked by the test compound in animal under investigation. Moreover it is a tool for calculating therapeutic index of a lead compound.

For a positive judgement on safety of a lead compound animal toxicity study is extreamely important and to find adequate potential for development into pharmacological compound.

The present study reveals the acute and sub-acute toxicity profile of black cumin oil and barley water as a nanoemulsion form.

No morbidity and mortality were observed on 1.5+4ml/kg treated group throughout in the 14 days observation period. In this study, the results showed no unwanted effects in acute dose administration groups. This indicates that the LD50 was found to be, greater than that of higher dose of administration. The sub-acute dose was selected based on the rats LD 50 value, which is greater than that of acute oral single dose.

In repeated dose 28- days oral toxicity study, there were no treatment related side effects were noticed in all the *NSO+BW* treated animals. The body weight changes are the markers of adverse effects of the drugs and chemicals. If the body weight loss occurred in more than 10% of the initial body weight of the animal will be considered as statistically significant.

There were no significant differences in body weight gain and organ weight of both control and treated groups of both sexes. Physiological and pathological state of an animal is also explained by its organ weight. The liver, heart, kidney, spleen and lungs are the primary organs of the animal affected by the toxic metabolic reactions.

It is also important to measure the food and water intake during the repeated dose toxicity determination. The proper intake of the supplements is necessary to evaluate the physiological status of the animal. Food and water consumption also did not affect abnormally during the experiment period. Thus, the results indicates there was no interruption in the diet metabolism.

Changes in the hematological and biochemical parameters have a great indicative value for toxicity determination in preclinical study. There is no specific alterations in the hematological parameters (platelet count, RBC count, WBC count and HB etc) during the repeated dose study. SGOT and SGPT (transaminases) are key indicators of liver functions and they are considered as biomarkers to conclude probable toxicity of the testing product. Destruction of liver parenchyma cells will normally cause the elevated level of these enzymes in blood. In this study there were no significant changes observed in AST and ALT in control and treatment groups, which reveals that the formulation didn't affect the liver physiology.

Total protein level and total bilirubin level in blood also indicates the behavior of liver function. Between the experimental groups the unvaried ranges in the total protein and total bilirubin were noticed. The normal values of creatinine suggest that repeated administration of *NSO+BW* didn't cause any damage to the kidney also.

Histopathological studies furnish supportive evidence for biochemical and hematological results. No histopathologiacal alterations are shown in the various organs of the animal such as liver, kidney, brain and heart of the entire groups. Based on results, it is inferred that, formulated *NSO+BW* will not produce any observed adverse effect level.

Cardioprotective Effect[109-115]

Heart contains an abundant concentration of diagnostic marker enzymes like CPK, LDH and transaminases (AST & ALT) and once the heart is metabolically damaged, they releases its content into the extra cellular fluid (ECF). Isoproterenol (ISO), a synthetic catecholamine, has cardio toxic effects on the myocardium. The effects of ISO on heart are mediated through β1- and β2- adrenoceptors. Both β1- and β2- adrenoceptors mediate the positive inotropic and chronotropic effects to β-adrenoceptor agonists. ISPH is well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for the irreversible damage to the myocardium.

In the present study, Significant increase was noticed in the activities of cardiac markers like LDH, CK-MB, AST and ALT in plasma of ISPH-treated rats or increased concentration in serum confirm the onset of myocardial ischemia. Hence which may be the reflection of consequences of cellular injury due to lipid peroxides.

CPK is a muscle specific enzyme mainly for heart and brain; therefore, its increased concentration in serum is may be the result of myocardial injury, cardiac insufficiency, arrhythmias and myocardial infarction due to cytosolic Ca2+overload. Calcium is an essential factor in phospholipase-associated degradation of membrane phospholipids,which leads to damage of mitochondrial membrane, and diminish electron transport along with the leakage of lysosomal enzymes. Treatment with Calcium antagonists like nifedipine, β-adrenergic blocker- propranolol and lipid lowering drug like guggulsterone partially reversed the changes in sarcolemma enzymes and improve the Calcium uptake in the damaged heart.

The levels of CK-MB was found to be 123.47 ± 3.62 in Group I normal rats which increased to 174.25 ± 5.39 when the rats were treated with ISPH. Then the rats on treatment continuously with the *NSO+BW* (1+2 ml) for 30 days, showed a decrease in the values, which were found to be 134.25 ± 4.31, which were comparable with the standard drug

Propranolol treated group whose values were found to be 130.75 ± 4.50. The levels of LDH was found to be 739.75 ± 5.65 in Group I normal rats which increased to 1881.75 ± 13.22 when the rats are treated with ISPH. Then the rats on treatment continuously with the *NSO+BW* for 30 days, showed a decrease in the values, which were found to be 1025.12 ± 9.32, which were comparable with the *Propranolol* treated group whose values were found to be 942.47 ± 10.15.

Transaminase (AST and ALT) levels are a sensitive indicator of liver cell injury and are also correlates poorly with severity of cardiac disease also. AST is found in decreasing order of concentration in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leucocytes and erythrocytes. ALT is maximally concentrated in liver and is relatively less specific for other muscle injuries. Both the enzymes released into the body in increasing quantity when the tissues are damaged.

The levels of AST and ALT were found to be 135.22±4.66and 87.4±4.84in Group I normal rats which increased to 165.47±5.10and 151.1±5.61 when the rats were treated with ISPH respectively. Then the rats on treatment for 30 days continuously with the *NSO+BW* showed a decrease in the values, which was found to be 154.025±4.005 and 74.8±4.33for Group IV which is comparable with the standard drug *Propranolol* treated group whose values were found to be 126.27±5.38and 111.4±3.66 (Table 11). The elevated level of AST and ALT in the serum in Group III and Group IV suggested a potential protective effect of the *NSO+BW* against ISPH induced heart damage The tendency of these enzymes to return to near normal in *NSO+BW* administered group is a clear manifestation of the cardioprotective activity of the product.

Histopathological Evaluation

The evaluated histopathological studies suggested that ISO induced toxicity leads to serious changes in the histology of heart. The increased generation of lipid peroxidation products

and associated reactive oxygen species lead to distruct the integrity and other pathological changes in the heart.

The results of histopathological studies suggest that ISP toxicity leads to serious changes in the histology of heart. The increased formation of lipid peroxidation products and associated reactive oxygen species lead to collapse in membrane integrity and other pathological changes in heart.

The cardioprotective efficacy of a drug is predominantly depends on the ability of either reducing the pathological behavior, or maintaining the physiology of macro and micro level, which has been attributed by toxins. The membrane stabilizing property and suppression in peroxidase generation action of the herbal components from nigella sativa and hordium vulgare might be helpful in alleviating the pathological changes caused by the isoproterenol hydrochloride in heart

In-vitro Thrombolytic Effect [104-107]

One of the important pathogenesis of acute coronary syndromes and vessel injury is thrombosis, which leads to the adherence of platelets and subsequent platelet activation. Platelet activation is absolutely necessary for the coagulation cascade of blood when normal blood vessels are injured. However, the interactions between platelets and collagen can also cause circulatory disorders, such as thrombosis, atherosclerosis, and myocardial infarction etc. Inhibition of the platelet-collagen interaction might be a promising approach to the prevention of such thrombosis.

Herbal combined dietary consumption of nigella sativa fixed oil and barley water inhibits platelet aggregation *in vitro*; therefore, they may be used to treat or prevent some cardiovascular diseases.

CHAPTER -VII

SUMMERY AND CONCLUSSION

Development of Herbal nanoemulsion in this study were optimized using tween 80 with the addition of cosurfactant ethanol 96% by high energy method. The formulated nanoemulsion was translucent homogenous with yellowish brown in colour and the average globule size is below 1000 nm. The studied nanoemulsion plays a key role in the cardiovascular protection.

Treatment with single oral dose and repeated sub-acute doses didn't arise any toxic signs in body weights, feed and water intake , biochemical, hematological and histopathological result also. Therefore the formulation selected to assess cardiovascular proective activity.

In summary, it has been concluded from the biochemical and histopathological evidence that the the herbal combination of black cumin oil and barley water have a potential to inhibit the cardio toxic effects induced by ISPH and possesses a significant medicinal value in the prophylactic treatment of MI. Efforts are in advancement to isolate and characterize the active principle, which is responsible for the cardioprotective efficacy and thrombolytic activity of this valuable medicinal plants. The search for new pharmacological-active compounds for drug development is an important issue, as the trend toward using standardized plant compounds quality, safety and efficacy will continue. Therefore, all efforts have to be targeted to reveal the chemical-pharmacological profiles of herbal ingredients and fixed combinations and to rationalize their therapeutic application.

CHAPTER – VIII

BIBILIOGRAPHY AND WEBOGRAPHY

1. http://www.who.int/cardiovascular_diseases/en/

2. http://www.cvdf.org/.

3. World Health Organization. Prevention of cardiovascular disease: Guidelines for assessment and management of cardiovascular risk. Geneva, WHO, 2007.

4. World Health Organization. The global burden of disease: 2004 update. Geneva World Health Organization,2008.

5. World Health Organization. Global status report on noncommunicable diseases 2010. Geneva, WHO, 2010.

6. Mendis S et al. Report for the Pathobiological Determinants of Atherosclerosis in Youth (PBDAY) Research Group: Atherosclerosis in children and young adults: An overview of the World Health Organization and International Society and Federation of Cardiology Study on Pathobiological Determinants of Atherosclerosis in Youth Study (1985– 1995). Prevention and Control, 2005, 1:3–15.

7. Ross R. Mechanisms of disease: Atherosclerosis – an inflammatory disease. New England Journal of Medicine, 1999, 340:115–126.

8. Davis NE. Atherosclerosis: An inflammatory process. Journal of Insurance Medicine, 2005, 37:72–75.

9. Suchalatha S, Devi CSS. Effect of Arogh - A polyherbal formulation on the marker enzymes in isoproterenol induced myocardial injury. Indian Journal of Clinical Biochemistry 2004; 19:184-189.

10. Todd GL, Cullan GE, Cullan GM. Isoproterenol induced myocardial necrosis and membrane permeability alterations in the isolated perfused rabbit heart. Exp Mol Pathol 1980; 33:43-54.

11. Grimm, D., Elsner, D., and Schunkert, H., et al. (1998). Development of heart failure following isoproterenol administration in the rat: role of renin angiotensin system. Cardiovas Res 37, 91–10.

12. Singal, P. K., Beamish, R. E., and Dhalla, N. S. (1983). Potential oxidative Pathways of catecholamines in the formation of lipidperoxides and genesis of heart disease. Adv Exp Med Biol 161, 391–401.

13. Karlsberg RP, Cryer PE, Roberts R. Serial plasma catecholamine response early in the course of clinical acute myocardial infarction: Relationship to infarct extent and mortality. Am Heart J. 1981; 102: 24-29

14. Geng B., Chang L., Pan C., Qi Y., Zhao J.,Pang Y., Du J., Tang C. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. Biochem. Biophys. Res. Commun. 2004;318:756–763

15. Sasikumar CS, Shyamala Devi CS. Protective effect of Abana®, a poly-herbal formulation, on isoproterenol-induced myocardial infarction in rats. Ind J Pharmacol 2000; 32:198-201

16. Rathore, N., John, S., Kale, M., and Bhatnagar, D.: Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol. Res., 38, 297–303, 1998.

17. Sasikumar CS, Shyamala Devi CS. Protective effect of Abana®, a poly-herbal formulation, on isoproterenol-induced myocardial infarction in rats. Ind J Pharmacol 2000; 32:198-201.

18. Yogeeta SK, Gnanapragasam A, Kumar SS, Subhashini R, Sathivel A, Devaki T. Synergistic interactions of ferulic acid with ascorbic acid: Its cardioprotetive role during isoproterenol-induced myocardial infarction in rats. Mol Cell Biochem. 2006; 283: 139-46.

19. Francis Morris, William J Brady. ABC of clinical electrocardiography Acute myocardial infarction—Part I, Clinical review. BMJ volume 324 6 april 2002:831-834.

20. Jaffe AS, et al: Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol 2006; 48:1.

21. Vinay Kumar et al. Robbins and Cotran pathologic basis of disease. – 8th ed.

22. Robert K. Murray, et al. Enzymes: Mechanism of Action, Harper's Illustrated Biochemistry. 26th edition, 7 : 57.

23. Rodak BF. Hematology, Clinical Principles and Applications. 2nd ed. Philadelphia: W.B Saunders; 2002:609-753.

24. Harmening DM. Clinical Hematology and Fundamentals of Hemostasis. 3rd ed. Philadelphia: F.D. Davis; 1997:481-508.

25. Hoffmeister HM. Overview of the relevant aspects of the blood coagulation system-focus and cardiovascular hemostasis. Kongressbd Dtsch Ges Chir Kongr. 2001;118:572-575.

26. Stiene-Martin EA, Lotspeich-Steininger CA, Koepke JA. Clinical Hematology. Principles, Procedures, Correlations. 2nd ed. Philadelphia: Lippincott; 1998:599-611.

27. Richard Finkel et al. Chapter 20, Blood Drugs. Lippincott's Illustrated Reviews: Pharmacology, 4th Edition

28. Cushman M: Epidemiology and risk factors for venous thrombosis. Semin Hematol 2007; 44:62.

29. Zwicker J, et al: Cancer-associated thrombosis. Crit Rev Oncol Hematol 2007; 62:126.

30. Varki A: Trousseau's syndrome: multiple definitions and multiple mechanisms. Blood 2007; 110:1723.

31. Wander GS, Chhabra ST. Critical Analysis of various Drugs used for Thromboly HC therapy in Acute Myocardial Infarction. In: Muruganathan A (ed). Medicine update, Volume 23. New Delhi: Jaypee Brothers Medical Publishers; 2013.pp109-16.

32. The GUSTO Investigators. An international randomized trial and comparing thrombolytic strategies for acute myocardial infarction. N Engl J Med. 1993;329:673– 682.

33. Grines CL, Browne KF, Marco J, Rothbaum D, Stone GW, O'Keefe J, Overlie P. Comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction: the Primary Angioplasty in Myocardial Infarction Study Group. N Engl J Med. 1993;328:673– 679.

34. Scharfstein JS, Abendschein DR, Eisenberg PR, George D, Cannon CP, Becker RC,. Sobel B, Cupples LA, Braunwald E. Usefulness of fibrinogenolytic and procoagulant markers during thrombolytic therapy in predicting clinical outcomes in acute myocardial infarction. Am J Cardiol. 1996;78:503–510.

35. 35.Rapold HJ. Promotion of thrombin activity by thrombolytic therapy without simultaneous anticoagulation. Lancet. 1990;1:481– 482.

36. Eisenberg PR, Miletich JP, Sobel BE. Factors responsible for the differential procoagulant effects of diverse plasminogen activators in plasma. Fibrinolysis. 1991;5:217–224.

37. Goto S, Kawai Y, Abe S, Takahashi E, Handa S, Ogawa S, Watanabe K, Hori S, Ikeda. Y. Serial changes in coagulant activities after thrombolytic therapy for acute myocardial infarction. Angiology. 1994;45:273–281.

38. Eisenberg PR, Sobel BE, Jaffe AS. Activation of prothrombin accompanying thrombolysis with recombinant tissue-type plasminogen activator. J Am Coll Cardiol. 1992;19:1065–1069.

39. Rapold HJ, de Bono D, Arnold AER, Arnout J, de Cock F, Collen D, Verstraete M. Plasma fibrinopeptide A levels in patients with acute myocardial infarction treated with alteplase. Circulation 1992;85: 928–934.

40. Baglin TP, Luddington R, Jennings I, Richards EM. Thrombin generation and myocardial infarction during infusion of tissue-plasminogen activator. Lancet. 1993;341:505. Abstract.

41. Merlini PA, Bauer KA, Oltrona L, Ardissino D, Spinola A, Cattaneo M, Broccolino M, Mannucci PM, Rosenberg RD. Thrombin generation and activity during thrombolysis and concomitant heparin therapy in patients with acute myocardial infarction. J Am Coll Cardiol. 1995;25:203–209.

42. Owen J, Friedman KD, Grossman BA, Wilkins C, Berke AD, Powers ER. Thrombolytic therapy with tissue plasminogen activator or streptokinase induces transient thrombin activity. Blood. 1988;72:616–620.

43. Hoffmeister HM, Jur M, Wendel HP, Heller W, Seipel L. Alterations of the coagulation, the fibrinolytic, and the kallikrein-kinin system in the acute and postacute phase in patients with unstable angina pectoris. Circulation. 1995;91:2520 –2527.

44. Hoffmeister HM, Ruf M, Wendel HP, Heller W, Seipel L. Streptokinaseinduced activation of the kallikrein-kinin system and of the contact phase in patients with acute myocardial infarction. J Cardiovasc Pharmacol. 1998;31:764 –772.

45. Huber K, Rose D, Resch I. Circadian fluctuations of plasminogen activator inhibitor and tissue plasminogen activator levels in plasma of patients with unstable coronary artery disease and acute myocardial infarction. Thromb Haemost. 1988;60:372–376.

46. Gram J, Kluft C, Jespersen J. Depression of tissue plasminogen activator (t-PA) activity and rise of t-PA inhibition and acute phase reactants in blood of patients with acute myocardial infarction (AMI). Thromb Haemost. 1987;58:817– 821.

47. Rennie JA, Ogston D. Changes in coagulation factors following acute myocardial infarction in man. Haemostasis. 1976;5:258 –264.

48. Gidron E, Margalit R, Oliven A, Shalitin Y. Effect of myocardial infarction on components of fibrinolytic system. Br Heart J. 1977;39: 19–24.

49. Sharma H,Chandola HM,Singh G,Basisht G.Utilization of Ayurveda in health care: an approach for prevention, health promotion, and treatment of disease;Part 2-Ayurveda in primary health care.J Altern Complement Med.2007 Dec;13(10):1135-50.

50. V. P. Kamboj. Herbal medicine: Current Science, Vol. 78, No. 1, 10 January 2000

51. Vickers A and Zollman C (1999). ABC of complementary medicine: herbal medicine. BMJ, 319, 1050 -3.

52. WachtelGalor S, Benzie IFF. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 1. PubMed NCBI 3/21/2016.

53. Mahmood ZA, Mohammad S, Mahmood SBZ, Karim MA. Herbal treatment for cardiovascular disease the evidence based therapy. Pak. J. Pharm. Sci 2010;23(1):119 124

54. Salah N, Miller NJ, Paganga G et al. Polyphenolics flavonols as scavenger of aqueous phase radicals and as chain breaking antioxidants. Arch. Biochem. Biophys 1995;2:339-346.

55. Hertog MGL, Sweetnam PM, Fehily A et al. Antioxidant flavonols and ischemic heart disease in a welsh population of 95 men: the caerphilly study. Am. J. Clin. Nutr 1997;65:1489-1494.

56. Jingjing Yu, Michel Weïwer, Robert J. Linhardt and Jonathan S. Dordick. The Role of the Methoxyphenol Apocynin, a Vascular NADPH Oxidase Inhibitor, as a Chemopreventative Agent in the Potential Treatment of Cardiovascular Diseases. Current Vascular Pharmacology, 2008;6:204-217

57. Ackerman RT, Mulrow CD, Ramirez G et al. Garlic shows promise for improving some cardiovascular risk factors. Arch Intern Med 2001;161:813-824.

58. Stevinson C, Pittle MH, Ernst E.Garlic for treating hypercholesterolemia. A meta-analysis of randomized clinical trials. Ann Intern Med 2000;133:420-429.

59. Bordia A. Effect of garlic on human platelet aggregation in vitro. Atherosclerosis 1978;30:355–360.

60. Bordia A, Bansal HC, Arora SK et al.Effect of the essential oils of garlic and onion on alimentary hyperlipemia. Atherosclerosis 1975;21:15-19.

61. Bordia A, Verma SK. Srivastava KC. Effect of garlic (Allium sativum) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. Prostaglandins Leukot Essent Fatty Acids 1998;58:257-263.

62. Mohammad SF, Woodward SC. Characterization of a potent inhibitor of platelet aggregation and release reaction isolated from allium sativum (garlic). Thromb Res 1986;44:793– 806.

63. Makheja AN, Vanderhoek JY, Bailey JM. Inhibition of platelet aggregation and thromboxane synthesis by onion and garlic. Lancet 1979;1:781.

64. Lawson LD, Ransom DK, Hughes BG. Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb Res 1992;65:141-156.

65. Apitz-Castro R, Cabrera S, Cruz MR et al. Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. Thromb Res 1983;32:155-169.

66. Ravinderpal Kaur , Kushwah A.S , Parminder Kaur. Status of Herbal Drugs in Cardiovascular Research: A Review: IJPBR, Vol. 1(1); March, 2013.

67. Makheja AN, Bailey JM. Antiplatelet constituents of garlic and onion. Agents Actions 1990;29:360-363.

68. Hertog MG, Feskens PC, Hollman PC et al. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007-1011.

69. Aviram M, Eias K. Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. Ann Nutr Metab 1993;37:75-84.

70. Fuhrman B, Buch S, Vaya J et al. Licorice extract and its major polyphenol glabridin protect lowdensity lipoprotein against lipid peroxidation: in vitro and ex vivo studies in humans and in atherosclerotic apolipoprotein Edeficient mice. Am J Clin Nutri 1997;66:267-75.

71. Teissedre PL, Frankel EN, Waterhouse AL et al. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. J Sci Food Agric 1996;70: 55-61.

72. Nawal.H.Al.Bahtiti. Chemical Investigation and Preservative Effect of Jordanian Nigella sativa Seed Oil on Date Paste. International Journal of Research Studies, in Biosciences (IJRSB) Volume 3, Issue 4, April 2015, PP 120-124.

73. Sabira Sultana, Hafiz Muhammad Asif, Naheed Akhtar, Asif Iqbal, Haleema Nazar, Riaz Ur Rehman. Nigella sativa: Monograph. Journal of Pharmacognosy and Phytochemistry, 2015; 4(4): 103-106.

74. Saha Rajsekhar, Bhupendar Kuldeep. Pharmacognosy and Pharmacology of Nigella Sativa – A Review. IRJP 2011, 2 (11), 36-39.

75. Daba MH, Abdel-Rahman MS. Hepato protective activity of thymoquinone in isolated rat hepatocytes. Toxicol Lett 1998;95:23-9.

76. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol 2001;110: 239-51

77. M. Murugesan, M. Ragunath, T. Prabu, S. Nadanasabapathi, M. Sakthivel, V. Manju. Protective role of black cumin (Nigella sativa) on isoproterenol induced myocardial infarction in rats: International Journal of Pharmacology and Clinical Sciences June 2012 Vol.1 Issue 2 45-53.

78. Saha Rajsekhar, Bhupendar Kuldeep. Pharmacognosy and Pharmacology of Nigella Sativa – A Review. IRJP 2011, 2 (11), 36-39.

79. The biology of hordium vulgare l.(barley), department of health and ageing office of the gene technology regulator, Australian govt.

80. Briggs, DE. (1978), barley, chapman and hall ltd, London.

81. Gomez – Macpherson, H.(2001). Hordeum vulgare.

82. Ravinderpal Kaur , Kushwah A.S , Parminder Kaur. Status of Herbal Drugs in Cardiovascular Research: A Review. IJPBR, Vol. 1(1); March, 2013.

83. Francis Morris, William J Brady. ABC of clinical electrocardiography, Acute myocardial infarction—Part I. BMJ 2002;324:831–4.

84. Marwa A. Ahmed, Khaled M. A. Hassanein. Cardio protective effects of Nigella sativa oil on lead induced cardio toxicity: Anti inflammatory and antioxidant mechanism. J. Physiol. Pathophysiol, vol.4(5), pp.72-80,dec,2013.

85. Azar Hosseini, Reza Shafiee-Nick, Seyed Hadi Mousavi. Combination of Nigella sativa with Glycyrrhiza glabra and Zingiber officinale augments their protective effects on doxorubicin-induced toxicity in h9c2 cells. Iran J Basic Med Sci, Vol. 17, No. 12, Dec 2014.

86. M. Kaleem, d kirmani, m asif, q ahmed, bilquees bano. biochemical effect of nigella sativa linn. seeds in diabetic rats. Indian journa of experimental biology. Vol.44.sept 2006.pp.745-748.

87. Huda kaatabi,Abdullah o.bamosa, fatma m. hebda, abdulmohsen h. al. elq, ali I,al-sultan. Favorable impact of nigella sativa seeds on lipid profile in type-2 diabetic patients. journal of family and community medicine, dec. 2012, vol 19,issue 3,155-161.

88. A.A. Abou gabal, A.E. Essawy, A.M. Abdel-Moneim, S.S. Hamed, A.A. Elzergy. The protective effect of black seed (Nigella sativa) against carbon tetrachloride-induced chromosomal aberrations and ultrastructural changes of bone marrow cells. Arab J. Biotech., Vol. 10, No. (2) July (2007):275-288.

89. Amr A. Rezq. Effects study of nigella sativa, Its oil and their combination with Vitamin E on oxidative stress in rats. American Journal of Applied Sciences 11 (7): 1079-1086, 2014.

90. Ahmed Jasim Mohammad Role of Black Seed Oil (Nigella sativa) and Olive Oil Combination on Serum Rabbit Treated with Cholesterol. International Journal of Science and Research (IJSR). Volume 4 Issue 7, July 2015, pp 654-659.

91. Neelam Chaturvedi, Parul Sharma, Kalpana Shukla, Rachna Singh and Sachdev Yadav.Cereals Nutraceuticals, Health Ennoblement and Diseases Obviation: A Comprehensive Review. Journal of Applied Pharmaceutical Science 01 (07); 2011: 06-12.

92. Vaclav Vetvicka*, Jana Vetvickova.Physiological Effects Of Different Types Of B-Glucan. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2007, 151(2):225–231.

93. Mokhtar I. Yousef, Medhat Haroun, Mohamed H. El-Masry and Reham EA. Ateia. Biochemical and Immunological Study on the Effects of Barley and it Components as Hypoglycemic Agents in Diabetic Rats American Journal of Biochemistry and Biotechnology 2 (1): 1-8, 2006.

94. Saima Gul, Sagheer Ahmed, Nurolaini Kifli, Qazi Tahir Uddin, Nafisa Batool Tahir, Abrar Hussain,Hawa ZE Jaafar, Marius Moga and Muhammad Zia-Ul-Haq. Multiple pathways are responsible for Anti-inflammatory and Cardiovascular activities of Hordeum vulgare L. Journal of Translational Medicine 2014, 12:316.

95. Joan M. Conway and Kay M. Behall. Health Effects of Barley Consumption.USDA

96. Inas E Darwish, Hala M Maklad, Iman H Diab.Behavioral and neuronal biochemical possible effects in experimental induced chronic mild stress in male albino rats under the effect of oral barley administration in comparison to venlafaxine. Int J Physiol Pathophysiol Pharmacol 2013;5(2):128-136.

97. Saurabh Arjariya et al. investigate the toxicological effect on aqueous extract of terminalia catappa linn. in rat. Int. J. Res. Dev. Pharm. L. Sci. August - September, 2013, Vol. 2, No. 5, pp 596-601.

98. Rabiatul et al. Assessment of Potential Toxicological Effects of Cinnamon Bark Aqueous Extract in Rats. International Journal of Bioscience, Biochemistry and Bioinformatics. Volume 5, Number 1, January 2015.

99. Prasanth kumar. M, Suba. V, Remireddy. B, srinivas Babu. P. Acute and Sub Acute (28-day) Oral toxicity studies of Ethanolic Extract of Celtis Timorensis Leaves in Rodents. Global Journal of Medeical Research: B Pharma, Drug Discovery, Toxicology and medicine. Vol 14, Issue 1, 2014.

100. Behera Saiprasanna, S. Manohar Babu, Y. Roja Ramani, Choudhury Prasanta Kumar, Panigrahi Rajeshree. Cardioprotective effect of pongamia pinnata Hydro-alcoholic leaf extract against Isoproterenol induced myocardial infarction In wistar albino rats. International Journal of Medical and Pharmaceutical Sciences (IJMPS) Vol 2 issue 3, pg 1-15.

101. Abi Beaulah G, Mohamed Sadiq A, Sivakumar V and Jaya Santhi R. Cardioprotective activity of methanolic extract of Croton sparciflorus on isoproterenol induced myocardial infarcted wistar albino rats. Journal of Medicinal Plants Studies 2014; 2(6): 01-08.

102. PakutharivuThangarajan, Anitha Anumanthan, Usha Venkatachalam, Sharmila Sivakumar, Chitra Somaskandan.Cardioprotective Activity of Pithecellobium Dulce Fruit Peel on Isoproterenol-Induced Myocardial Infarction in Rats. Int. J. Pharm. Sci. Rev. Res., 30(1), January – February 2015; Article No. 24, Pages: 133-136.

103. Alam Firoj, Siddiqui H .H. Cardioprotective Activity of Terminelia Belerica on Isoprenaline Induced Myocardial Necrosis in Rat. AJCPR, 2014, Vol.2(2): 127-136.

104. E. Sathyapriya, v. Velpandian, j. Anbu and ashwini anjana. In vitro anti platelet aggregation activity and Thrombolytic activity of cheenalinga chendhuram. International journal of life science and pharma research. Vol 2/Issue 4/Oct-Dec 2012.pp 51-55.

105. Vuyyuri Bhaargavi, Sindhuja s. Thrombolytic activity of herbal mixture containing aqueous crude extracts. Int. J. Res. Dev. Pharm. L. Sci. June - July, 2016, 5(4), 2264-2268.

106. A. Elumalai. M. China Eswariah, Vidhyulatha chowdary, Ranjith Kumar, Anusha M, Naresh K. screening of thrombolytic activity of Bogainvillea glabra leaves extract by in-vitro. Asian J. Res. Pharma. Sci. 2012; Vol 2: Issue 4, Pg 134-136.

107. Mubarak yusuf Ibrahim et al. evaluation of thrombolytic and cytotoxic activity of an ornamental medicinal plant: Byttneria pilosa. American Journal Of Biomedical Research, 2015, Vol. 3, No 2, 35-38.

108. Behera Saiprasanna, S. Manohar Babu, Y. Roja Ramani, Choudhury Prasanta Kumar, Panigrahi Rajeshree. Cardioprotective effect of *pongamia pinnata* Hydro-alcoholic leaf extract against Isoproterenol induced myocardial infarction In wistar albino rats. International Journal of Medical and Pharmaceutical Sciences (IJMPS) Vol 2 issue 3, pg 1-15.

109. Abi Beaulah G, Mohamed Sadiq A, Sivakumar V and Jaya Santhi R. Cardioprotective activity of methanolic extract of *Croton sparciflorus* on isoproterenol induced myocardial infarcted wistar albino rats. Journal of Medicinal Plants Studies 2014; 2(6): 01-08.

110. PakutharivuThangarajan, Anitha Anumanthan, Usha Venkatachalam, Sharmila Sivakumar, Chitra Somaskandan.Cardioprotective Activity of Pithecellobium Dulce Fruit Peel on Isoproterenol-Induced Myocardial Infarction in Rats. Int. J. Pharm. Sci. Rev. Res., 30(1), January – February 2015; Article No. 24, Pages: 133-136.

111. Alam Firoj, Siddiqui H .H. Cardioprotective Activity of *Terminelia Belerica* on Isoprenaline Induced Myocardial Necrosis in Rat. *AJCPR, 2014, Vol.2(2): 127-136.*

112. Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. Free Radical Biol Med 1994; 16:845-50.

113. Herman EH, Zhang J, Chadwick DP, Ferrans Okinaka S, Kumogai H, Ebashi S, Sugita H, Momoi H, Toyukura Y. Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular diseases. J. Biol. Chem 1961; 4:520-525.

114. Urizar NL, Liverman AB, Dodds DNT, Silva FV, Ordentlich P, Yan Y *et al*. A natural product that lowers cholesterol as an antagonist ligand for FXR. Science 2002; 296:1703-1706.

115. Kumar PBJR, John De Britto A, Guru Manekandan A. Preliminary phytochemical evaluation of a few vital raw drugs used in siddha medicine. International journal of institutional pharmacy and life sciences. 2014; 4(2).

.